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INFECTION OF BARLEY BY USTILAGO NUDA THROUGH SEED INOCULATION [1]

By W. H. TISDALE, *Pathologist*, and V. F. TAPKE, *Associate Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Floral infection of barley (*Hordeum sativum* L.) by *Ustilago nuda* (Jens.) Kell. and Sw., as well as floral infection of wheat (*Triticum vulgare* Vill.) by *Ustilago tritici* (Pers.) Jens., has been considered an established fact for many years. There was considerable confusion regarding the infection of cereals by smut fungi before the different species of these fungi were identified and described. Since these differences were found steady progress has been made in the discovery of the morphological and physiological differences of the smut fungi, of their host relationships, and of the control of the smuts caused by them.

Since the discovery of floral infection (*16*) [2] the hot-water seed treatment has been considered the only means of controlling the loose smuts of wheat and barley. Recently, however, formaldehyde and some of the organic mercury compounds have been found to control the loose-smut of barley in certain varieties (*13*, *20*, *21*, and *22*). This led to the belief that there remained important facts to be learned regarding the infection of barley by *Usitlago nuda*.

In the autumn of 1922 the senior writer planned to study the possibility of seedling infection by this fungus. The results of the first experiment were so striking that a number of experiments, in which nine varieties of barley were used, was conducted during the past season, 1923–24. The results of these studies were even more striking than those of the previous year.

This paper gives a brief review of the literature dealing with floral infection of wheat and barley by their respective loose-smut fungi, presents the data of the writers' recent studies showing that previous investigators have been confused regarding the infection of barley by *Ustilago nuda*, at least to a certain extent, and that the fungus infects the seedlings, at least of certain varieties of barley, and causes severe injury to the seedlings when heavily infected.

HISTORY OF FLORAL INFECTION

Hoffman (*10*), in 1866, long before the discovery of the difference in the fungi causing the smuts of barley, mentioned the possibility of floral infection. He was studying barley smut principally but did some work with the smuts of wheat and oats.

In 1888, Jensen (*12*), after several years of study of infection of barley and oats by smut (*Ustilago segetum*), made the following statement:

> Since we have seen that spores adhering to the exterior of the grain do not to any appreciable extent cause the infection of the crop, it follows that this must take place by means of those spores which succeed in entering the space between the "cosh" or husk and the kernel.

He then suggested two possibilities for infection; one being floral infection by direct germination of the spores immediately after they enter the flowers, and the other being the infection of the seedlings by spores which remained quiescent between the husk and the kernel until the seed was sown and then germinated. He says that his experiments of 1887 point to the latter. He found that by removing the hulls (glumes) of barley and oats and inoculating the seed, higher percentages of infection could be obtained.

After a large part of these experiments was concluded Jensen found that there were two smuts of barley and described them as *Ustilago segetum*, variety *hordei nuda*, "the naked smut," and *Ustilago segetum*, variety *hordei tecta*, "the covered smut." Previous to this, he had assumed that the two were identical, *U. segetum* DC. After this discovery he says:

> In the experiment quoted above with barley smut the variety *tecta* only was used for infecting the bare kernels.

At about the same time, 1888, Brefeld (*1*) failed to obtain infection of barley by inoculating the seed and young seedlings with germinated and ungerminated spores. He then obtained smutty barley heads from Japan and found that the spores from these

[1] Received for publication, Aug. 25, 1924. Issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 283–284.

heads germinated and formed mycelium but no conidia. Conidia were produced by the form previously used. He called the form from Japan *Ustilago hordei*. This was evidently the form which is now known as *U. nuda* which causes loose-smut. Brefeld evidently made his infection studies with the fungus of covered smut, *Ustilago hordei*. He makes no mention in this paper of further infection studies after discovering this difference in the barley smut fungi.

In 1899 Kellerman and Swingle (*14*), in a study of the barley smuts, described the two species *U. hordei* (Pers.) Kell. and Sw., covered smut, and *U. nuda* (Jens.) Kell. and Sw., loose-smut. They cited Jensen's statements concerning floral infection but said, "This view requires confirmation."

Maddox (*16*) concluded from carefully conducted experiments that the loose smuts of wheat and barley could not be produced by smutting the seed or by mixing spores with the soil. He says:

> Artificially smutted is putting the smut germs on the ovary about the time the pollen is ripe, which will always reproduce the disease the following year.

According to Hori (*11*), K. Yamada in 1896 and S. Nakagawa in 1897 produced loose-smut in wheat by inoculating the flowers with mature spores of *Ustilago tritici*.

Brefeld (*2*) in 1903 discussed methods of floral inoculation of wheat and barley with the loose-smut spores. He concluded that infection takes place through the flower. Again in 1905 Brefeld (*3*) published, in detail, methods used in attempting to infect barley with *Ustilago nuda* by inoculating the flowers and seed with spores and by inoculating the young seedlings with germinated spores. In one experiment he obtained 1 per cent infection where the seedlings were inoculated with germinating spores, while two-rowed barley treated similarly remained smut free, as did the control. This was the only indication of seedling infection. Brefeld's results led him to conclude, however, that—

> infection in the blossom is the predominant form of infection of the host plants, if not the only one.

Hecke (*8*) produced floral infection in barley in 1904, and in 1905 he (*9*) figures the mycelium in the scutellum of barley. He adheres to Brefeld's terminology and calls the loose-smut fungus *Ustilago hordei*.

Hori (*11*) in 1907 states that he obtained floral infection by *Ustilago tritici* and *U. nuda* as early as 1900. After discussing the findings of Brefeld and Hecke he makes the following statement:

> Hence it is now clear that at least *U. tritici*, *U. nuda* and *U. hordei* may naturally infest the respective host plants by the flower infection.

Hori evidently was referring to the loose-smut of barley both as *U. nuda* and *U. hordei*. Brefeld (*1*) called the loose-smut fungus *U. hordei* when he first discovered that there were two barley smuts. Hori was probably confused by this difference in nomenclature.

Falck (*5*) in 1908 and Lang (*15*) in the following year studied the infection of wheat by *Ustilago tritici* cytologically and showed the mycelium in the stigma and the young ovaries and showed it ramifying in the cells of the embryo at various stages of development.

Freeman and Johnson (*7*) in 1909 studied the loose smuts of wheat and barley. They inoculated the flowers at different stages of maturity and found that infection takes place from the time when the stamens are still green to the time when the ovary is one-third its mature size. They found the optimum time for infection to be when the flower is in full bloom or when the ovary is just commencing to develop after fertilization.

Brioli (*4*) in 1910 studied floral infection in wheat and barley and confirmed the work of previous investigators. He illustrates the mycelium in the scutellum of wheat and says that he found mycelium in the scutellum of one variety of barley but not in the other varieties:

> Nur bei Körnern der Niederbayerischen Gerste habe ich Mycel gefunden. Bei den anderen Gersten nicht.

He attributes the lack of mycelium in the kernels to resistance of the host.

PRESENT INVESTIGATIONS

SEEDLING INFECTION

For many years after the discovery of the hot-water seed treatment by Jensen (*12*) this method was recommended as being the only treatment that would control the loose-smuts of wheat and barley. In 1914, Johnson (*13*) reported the control of loose-smut of barley by treating the seed with formalin. Tisdale, Taylor, and Griffiths (*21*) in 1923, obtained satisfactory control of loose-smut of barley by treating the seed with formaldehyde and with chlorophol, an organic mercury compound. Six varieties of barley were included in this experiment. Since that time Semesan, Corona 620,

Uspulun and Germisan (organic mercury compounds) have been found to control the loose-smut of barley (*20, 22*).

These results were surprising in view of the claims and apparent proof of floral infection of barley by *Ustilago nuda*. At the same time, it led to the belief that it might be possible that previous investigators were misled, in some way, regarding infection of barley by the loose-smut fungus. Of course, it seemed entirely possible that floral infection might take place and the fungus mycelium remain in the superficial cells of the kernel where it could be destroyed by disinfectants which penetrate the seed coat.

Tisdale (*19*) found that by inoculating dehulled[3] barley kernels with spores of the covered smut fungus *Ustilago hordei*, high percentages of infection could be obtained, while often little or no infection could be secured by inoculating the hulled seed. In the fall of 1922, while conducting the experiments on covered smut, the senior writer inoculated some dehulled seed of Tennessee Winter and Nakano Wase barleys with spores of *U. nuda* and sowed it on a bench in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. Dehulled-uninoculated, hulled-inoculated, and hulled-uninoculated seed of both varieties also was sown. The seed for this and subsequent experiments was dehulled by carefully removing the glumes with a sharp-pointed knife or scalpel. The basal end was first broken and the glumes stripped off. Nakano Wase remained smut-free in all experiments. The dehulled-inoculated seed of Tennessee Winter produced 25 per cent of loose-smut, or 5 plants in a total of 20, while only 1 plant in 22 from the dehulled-uninoculated and 1 in 20 from the hulled-inoculated seed produced loose-smut. (Table I.)

The occurrence of 25 per cent of loose-smut in plants from inoculated-dehulled seed appeared to be significant. This led to plans for further experiments in which both floral and seedling infection could be studied. Six varieties of barley were used in these studies. Floral inoculations were made in the spring of 1923 on barleys growing in the greenhouse. Each flower was carefully inoculated by opening the glumes with forceps and dusting the floral parts with fresh spores of *Ustilago nuda* from Tennessee Winter barley growing in the greenhouse. Flowers and seeds were inoculated in different stages and treated in different ways, as follows:

1.—Inoculated when anthers were green; no pollen shed.

2.—Inoculated when anthers were shedding pollen.

3.—Inoculated when pollen was recently shed, stigma plumose, and showing no signs of withering.

4.—Inoculation at tip of kernel when in the milk stage. The glumes were pulled back slightly, spores placed on the kernel, and the glumes replaced.

5.—Mature seed dehulled and sown without inoculation.

6.—Mature seed dehulled and inoculated by shaking thoroughly in an envelope with spores until almost black.

7.—Mature hulled seed sown without inoculation.

8.—Mature hulled seed inoculated.

Spore material used for inoculating the mature seed was collected from Tennessee Winter barley grown on Arlington Experiment Farm in 1923. The seed inoculated by the various methods given above was sown on a bench in the greenhouse on November 4, 1923. The seeds were sown about 1 inch deep and 2 inches apart in rows 7 inches apart. The results of these experiments are given in Table II.

TABLE I.—*Infection of barley seedlings by Ustilago nuda in the greenhouse in 1922–23*

Variety	Treatment	Number of plants		Percentage of smut
		Total	Smutted	
Tennessee Winter	Hulled-uninoculated	25	0	0
Do.	Hulled-inoculated	20	1	5.00
Do.	Dehulled-uninoculated	22	1	4.55
Do.	Dehulled-inoculated	20	5	25.00
Nakano Wase	Hulled-uninoculated	21	0	0
Do.	Hulled-inoculated	20	0	0
Do.	Dehulled uninoculated	24	0	0
Do.	Dehulled-inoculated	19	0	0

[3] Hulled = hulls not removed, and dehulled = hulls removed.

TABLE II.—*Infection of barley by Ustilago nuda in the greenhouse in 1923–24, by flower and seed inoculation* [a]

Variety	Type and stage of inoculation	Number of plants			Number of heads		
		Total	Smutted	Per cent of smut	Total	Smutted	Per cent of smut
Alaska	3. Pollen shed	81	3	3.70	233	13	5.58
Do	4. Milk stage	85	2	2.35	281	4	1.42
Do	5. Dehulled, uninoculated	15	0	0	41	0	0
Do	6. Dehulled, inoculated	47	36	76.60	188	148	78.72
Do	7. Hulled, uninoculated	30	0	0	115	0	0
Do	8. Hulled, inoculated	42	6	14.29	144	12	8.33
Greece	3. Pollen shed	64	1	1.56	210	1	0.48
Do	4. Milk stage	19	1	5.26	84	9	10.71
Do	5. Dehulled, uninoculated	29	0	0	101	0	0
Do	6. Dehulled, inoculated	49	31	63.27	142	80	56.34
Do	7. Hulled, uninoculated	23	0	0	69	0	0
Do	8. Hulled, inoculated	42	0	0	124	0	0
Han River	3. Pollen shed	5	0	0	14	0	0
Do	4. Milk stage	(b)	(b)	(b)	123	4	3.25
Do	5. Dehulled, uninoculated	11	0	0	44	0	0
Do	6. Dehulled, inoculated	33	29	87.88	146	132	90.41
Do	7. Hulled, uninoculated	33	2	6.06	110	3	2.73
Do	8. Hulled, inoculated	46	9	19.57	127	25	19.69
Nakano Wase	1-3. Anthers green to pollen shed	104	0	0	375	0	0
Do	4. Milk stage	65	0	0	284	0	0
Do	5. Dehulled, uninoculated	17	0	0	73	0	0
Do	6. Dehulled, inoculated	46	0	0	167	0	0
Do	7. Hulled, uninoculated	32	0	0	110	0	0
Do	8. Hulled, inoculated	35	0	0	127	0	0
Texas Winter	2-3. Pollen shedding to shed	72	5	6.94	187	19	10.16
Do	4. Milk stage	54	9	16.67	210	42	20.00
Do	5. Dehulled, uninoculated	9	1	11.11	21	3	14.29
Do	6. Dehulled, inoculated	14	14	100.00	114	114	100.00
Do	7. Hulled, uninoculated	35	0	0	154	0	0
Do	8. Hulled, inoculated	37	6	16.22	133	15	11.28
Wisconsin Winter	2-3. Pollen shedding to shed	79	4	5.06	274	9	3.28
Do	4. Milk stage	42	0	0	151	0	0
Do	5. Dehulled, uninoculated	26	0	0	89	0	0
Do	6. Dehulled, inoculated	40	21	52.50	143	73	51.05
Do	7. Hulled, uninoculated	21	0	0	75	0	0
Do	8. Hulled, inoculated	39	6	15.38	160	24	15.00

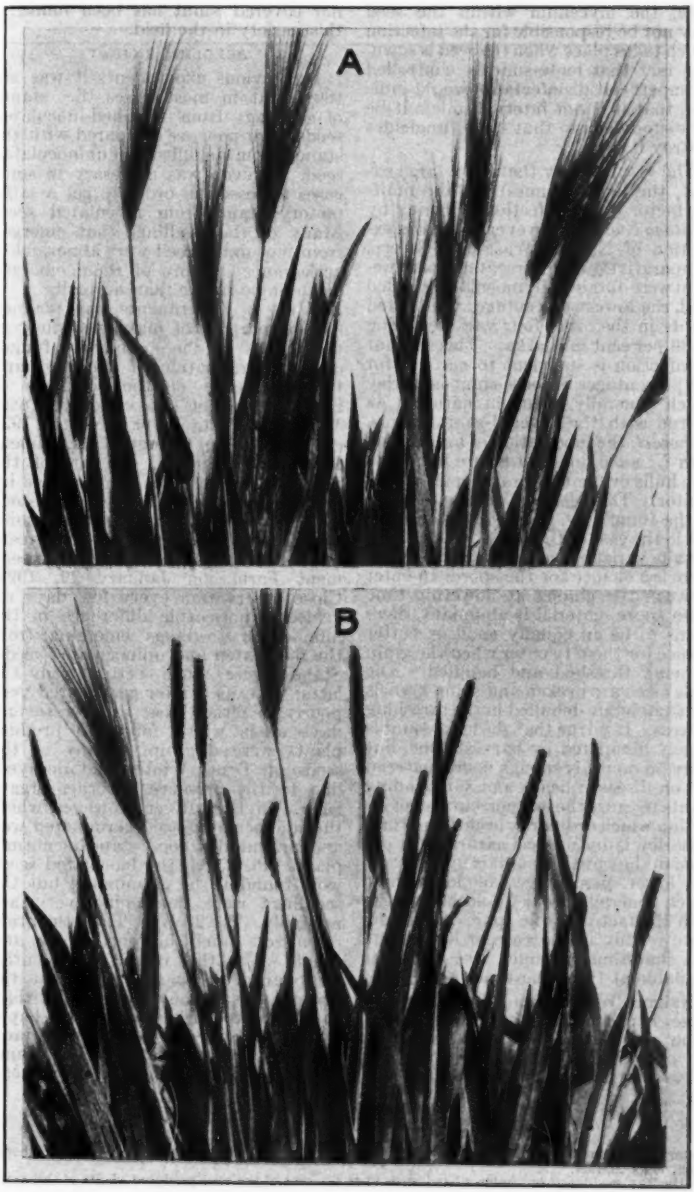
[a] The type of inoculation is referred to by number in the second column from the left in this table. The numbers refer to the explanations of time and method of inoculation given above.
[b] Not counted.

The smut records in Table II show strikingly that seedling infection takes place when the mature seed is dehulled and inoculated with spores of *Ustilago nuda* (Pl. 1, B). There evidently was a small amount of natural infestation in the seed used as two smutted plants occurred in one of the Han River controls and one plant in one of the Texas Winter controls. Other controls were smut-free (Pl. 1, A). The seed was not disinfected in any case as there was a possibility of injuring the vitality and interfering with the results.

Apparently some infection was obtained in some cases by dusting the spores on the stigma and ovary at flowering time and afterward. This appears to be true for all varieties except Han River and Nakano Wase, The percentages of infection, however, are not as high as have been obtained by previous investigators using this method of inoculation. As suggested by Jensen (*12*), there is a possibility that the flowers were infected, and also a possibility that the spores remained between the glumes and the kernel and germinated and produced seedling infection when the seed germinated. Microscopic examination three days after the flowers were inoculated showed that many of the spores had germinated within the flowers of Alaska barley. No infections were found. This limited examination does not prove, by any means, that infection does not take place. Neither does it prove that the ungerminated spores are not finally responsible for infection. Even though infection takes place in the flower, which seems entirely pos-

EXPLANATORY LEGEND FOR PLATE 1

Infection of Han River barley by *Ustilago nuda*. Plants grown on bench in greenhouse.
A.—Seed dehulled and uninoculated. All plants remained smut-free
B.—Seed dehulled and inoculated with spores of *U. nuda*; 87.88 per cent of the plants and 90.41 per cent of the heads were smutted.



Infection of Barley by Ustilago nuda

Plate 1

(For explanatory legend see p. 266)

sible, the mycelium within the seed may not be responsible for the infection which takes place when the seed is sown. The fact that loose-smut is controlled by superficial disinfectants would indicate that it is not internal unless it be very superficial so that these fungicides destroy it.

The results show that, to a large extent, the hulls (glumes) are the limiting factor in the infection of barley by *Ustilago nuda*. However, with the exception of Nakano Wase and Greece, comparatively high percentages of loose-smut were obtained by inoculating hulled seed, the lowest percentages of infected plants in the other four varieties being 14.29 per cent in Alaska. This amount of infection is sufficient to account for the percentages of loose-smut in barley which generally occur in nature. As is true with the covered-smut fungus, *U. hordei*, the inoculation of hulled seed with *U. nuda* does not insure infection. The hulls evidently are an important inhibitor. There may be different strains of the fungus, as Faris (*6*) has pointed out in the case of *U. hordei*, which infect certain varieties more readily. While there is a chance for the spores to enter between the glumes at flowering time when spore material is abundant, there seems to be an equally good or better chance for them to enter when the grain is being threshed and handled. The hulls often are broken and some kernels are completely dehulled in the threshing process. It is true that the loose-smut is largely dissipated by harvest time, but there no doubt is enough spore material left on diseased heads and surrounding plants to cause the low percentages of infection which ordinarily occur in nature. If barley is inoculated naturally by *U. nuda* in the same way as it is by *U. hordei* the lower percentages of loose-smut which generally occur would harmonize with the fact that the spores disappear more readily in the case of loose-smut and that there is much less inoculum available at threshing time

Nakano Wase remained smut-free in these experiments. It failed to become smutted with covered smut in the experiments of Tisdale (*19*). So far as the writers know, neither loose nor covered smut has been found in this variety in the field.

SEEDLING INJURY

In previous experiments it was noticed that in most cases the stands of seedlings from dehulled-inoculated seeds were poor as compared with the stands from dehulled and uninoculated seed. It even was necessary in some cases to reseed in order to get a satisfactory stand from inoculated seed. Many of the seedlings that emerged from inoculated seed were abnormal in appearance. Many of them emerged at an angle rather than normally.

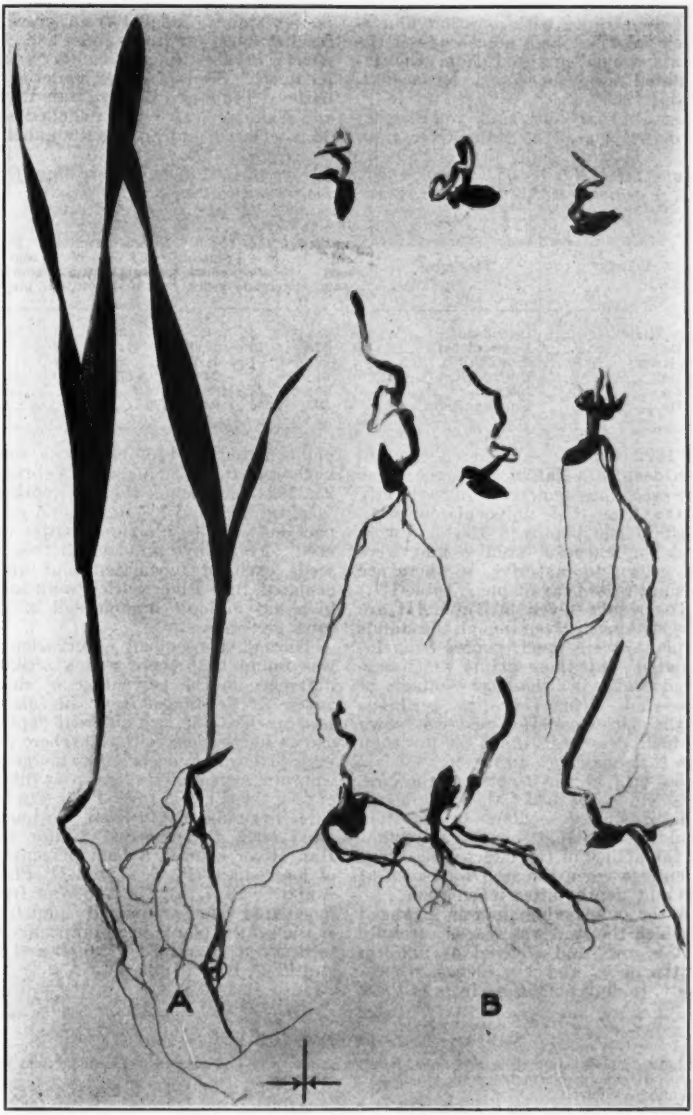
A set of experiments was planned for the purpose of making a study of the effects of the loose-smut fungus on the germination of the seed and the emergence of the seedlings of barley. Seed of two varieties, Texas Winter and Han River, from the 1921 crop grown on Arlington Experiment Farm was dehulled. A part of the dehulled seed of each variety was inoculated and sown and a part sown without inoculation. It was sown about 2 inches deep in soil on greenhouse benches at Arlington Experiment Farm, on January 29, 1924. Close observation every few days revealed a noticeable difference in the number of seedlings emerging from the inoculated and uninoculated seeds. Stands were much better from the latter. Texas Winter germinated very poorly in either case. After several days seeds which failed to produce plants were dug up. Many of the seeds of Texas Winter had decayed, due to the presence of other organisms. In Han River, however, where the uninoculated seed germinated well and produced a good stand of normal plants (Pl. 2, A), the inoculated seeds were found to be germinated but the seedlings were developing very abnormally (Pl. 2, B). The coleoptiles often were much shortened, thick, and tough. For this reason, apparently, they failed to open normally at the tip and the plumule was held within, causing it to curl into various shapes which prevented emergence. Many seedlings which did emerge came through the soil at an angle instead

EXPLANATORY LEGEND FOR PLATE 2

Injury to seedlings of Han River barley by *Ustilago nuda*. Seed dehulled and sown in soil on greenhouse bench. Jan. 29, 1924.

A.—Healthy seedlings from uninoculated seed; 93.44 per cent of the seed produced plants of this type, which were smut-free at maturity.

B.—Seedlings from inoculated seed infected by *U. nuda*. Only 11.29 per cent of the seed produced seedlings sufficiently normal to emerge. The remainder of the seeds germinated but the seedlings were too abnormal to emerge. The infected coleoptiles failed to open up normally and the seedlings became twisted in various ways.



Infection of Barley by Ustilago nuda

Plate 2

(For explanatory legend see p. 268)

of erect, as previously mentioned, and were yellowish in appearance. Some of these later developed a somewhat stiff appearance with a rather bluish-green color. A high percentage of the plants which matured from the inoculated seed developed loose-smut. (Table III.)

On February 13, 1924, another set of dehulled seed of Han River from the 1922 crop was sown about ¾ of an inch deep in a flat in the greenhouse. The seedlings emerged but slightly better from the uninoculated seed. Most of the plants in this flat were used for infection studies and were not grown to maturity, so there are no smut records available. (Table III.)

TABLE III.—*Effects of Ustilago nuda on the emergence of barley seedlings from dehulled seed, and the occurrence of loose-smut in the surviving plants*

Variety	Treatment	Number of seed sown	Number of plants emerged	Per cent of emergence	Number of plants matured	Number of plants smutted	Per cent of smutted plants
Texas Winter	Inoculated	56	3	5.36	3	3	100
Do	Uninoculated	57	20	35.09	11	0	0
Han River	Inoculated	62	7	11.29	5	4	80
Do	Uninoculated	61	57	93.44	57	0	0
Do	Inoculated	50	44	88.00			
Do	Uninoculated	50	46	92.00			

The results given in Table III are very striking. Even though the stands of plants were much poorer from inoculated seed, those plants which survived showed a high percentage of loose-smut. Emergence of seedlings in the flat where the seed was sown ¾ inch deep was much better than was the emergence from seed sown 2 inches deep on the benches. These results led to the belief that the degree of injury to the seedlings by infection would be indicated to a certain extent by the ability of the infected seedlings to emerge when the seed was sown at different depths after inoculation.

A set of experiments was arranged in which the seed was spaced carefully in the rows and covered at uniform depths of ¾ and 1½ inches, respectively, to determine the effects of these depths of sowing on the emergence of the seedlings. It seemed probable that the deeper sowing would produce poorer stands, judging from previous results, both as to stands and the effects of the fungus on the young seedlings. Seed of three varieties of barley—Tennessee Winter, Han River, and Nakano Wase—was dehulled and 25 inoculated and an equal number of uninoculated seed of each was sown in flats in the greenhouse on February 25, 1924, at each of the two depths—¾ of an inch and 1½ inches. A compact soil with little organic matter was used. Four days previous to this, 50 seeds each of inoculated and uninoculated Han River barley were sown in a flat of loose organic soil in the same greenhouse.

Through subsequent observation, it was found that there was a striking difference in the percentage of emergence of seedlings from inoculated seed sown at the two different depths, except in the loose soil, while there was very little difference in the emergence from uninoculated seed sown at different depths (Pl. 3, A to H, Pl. 4, A to D). The surviving plants from the inoculated seed of Tennessee Winter and Han River showed a high percentage of loose-smut (Pl. 5, A and C; Pl. 6, A and C; Pl. 7, A); Nakano Wase from inoculated seed remained smut-free, as did all the plants from uninoculated seed (Pl 5, B and D; Pl. 6, B and D, and Pl. 7, B). (Table IV.)

EXPLANATORY LEGEND FOR PLATE 3

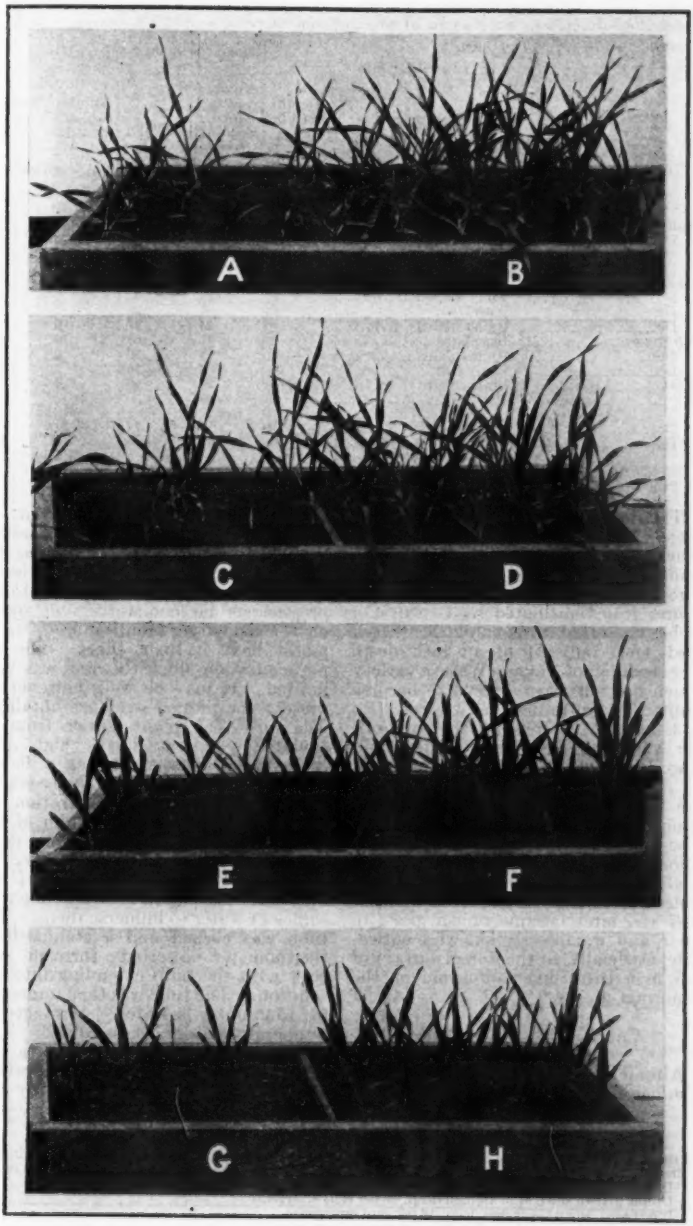
Infection of barley seedlings from dehulled seed by *Ustilago nuda*, and emergence of seedlings from seed sown at different depths in flats in the greenhouse on Feb. 25, 1924.

Tennessee Winter:

A.—Inoculated, sown ¾ of an inch deep; 56 per cent emergence.
B.—Uninoculated, sown ¾ of an inch deep; 88 per cent emergence.
C.—Inoculated, sown 1½ inches deep; 32 per cent emergence.
D.—Uninoculated, sown 1½ inches deep; 84 per cent emergence.

Nakano Wase:

E.—Inoculated, sown ¾ of an inch deep; 60 per cent emergence.
F.—Uninoculated, sown ¾ of an inch deep; 92 per cent emergence.
G.—Inoculated, sown 1½ inches deep; 28 per cent emergence.
H.—Uninoculated, sown 1½ inches deep; 92 per cent emergence.



Infection of Barley by Ustilago nuda

Plate 3

(For explanatory legend see p. 270)

99183—25†——2

TABLE IV.—*Effects of Ustilago nuda on the emergence of barley seedlings from dehulled-inoculated seed sown at two different depths, and the occurrence of loose-smut in the surviving plants*

Variety	Treatment	Depth sown in inches	Number seedlings from 25 or 50 seeds	Percentage of emergence	Number of plants smutted	Percentage of smut
			From 25 seeds			
Tennessee Winter	Inoculated	¾	14	56	11	78.57
Do	Uninoculated	¾	22	88	0	0
Do	Inoculated	1½	8	32	8	100.00
Do	Uninoculated	1½	21	84	0	0
Nakano Wase	Inoculated	¾	15	60	0	0
Do	Uninoculated	¾	23	92	0	0
Do	Inoculated	1½	7	28	0	0
Do	Uninoculated	1½	23	92	0	0
Han River	Inoculated	¾	17	68	14	82.35
Do	Uninoculated	¾	24	96	0	0
Do	Inoculated	1½	*4	16	2	100.00
Do	Uninoculated	1½	24	96	0	0
			From 50 seeds			
Do	Inoculated	¾	37	74	34	91.89
Do	Uninoculated	¾	47	94	0	0

* Two died.

The data in Table IV show that there was a marked reduction in emergence of seedlings from dehulled-inoculated seed. This was true for all three varieties. The stands were much poorer from inoculated seed sown 1½ inches deep than from similarly treated seed sown only ¾ of an inch deep. The seedlings of Nakano Wase, a variety which has not been known to become smutted, were injured as severely (Pl. 3, E and G) as those of the susceptible varieties, Tennessee Winter and Han River (Pl. 3, A and C and Pl. 4, A and C). The seedlings from the inoculated seed showed the same abnormalities as previously mentioned. They were twisted, and yellowish in color, and some emerged at an acute angle to the soil surface. After emergence the leaves appeared somewhat stiff and later became bluish green in color, and waxlike streaks were noticeable, especially on the dorsal surface of the first two leaves on some of the abnormal plants.

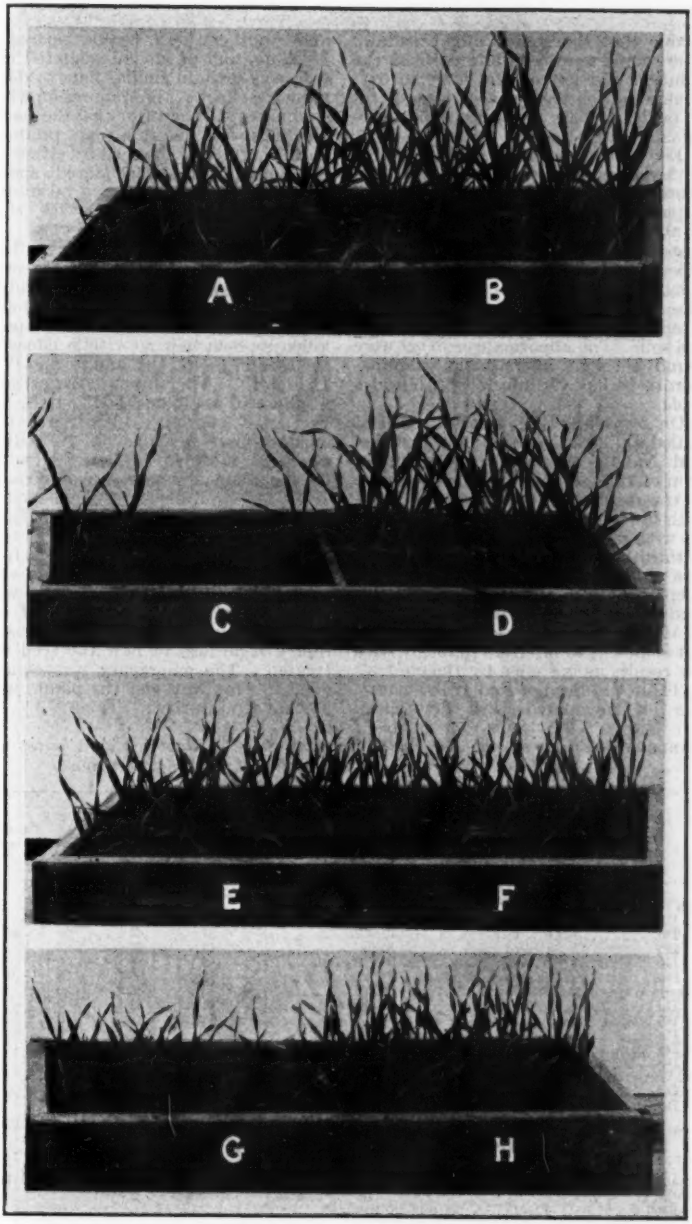
Cause of Seedling Injury

After it had been definitely shown that *Ustilago nuda* caused considerable injury to barley seedlings grown from dehulled-inoculated seed, the question arose as to whether this injury was due to infection and invasion of the tissues of the seedlings by the fungus or to the presence of large quantities of spores on the naked germinating seed, which might have a toxic effect. The experimentation along this line was very limited. It may be well, however, to present the meager evidence obtained. The fact that plants grown from inoculated seed produced high percentages of loose-smut was sufficient evidence that infection of the seedlings took place. Actual penetration of the seedlings by the mycelium of the fungus also had been found in cytologic studies to be discussed later.

A quantity of viable spores of *Ustilago nuda* were placed in a test tube with sufficient water to immerse them. The tube was corked and a standardized thermometer inserted through the cork with the bulb extending into the solution. The tube was then immersed for 15 minutes in water that ranged in temperature from 59 to 61° C. This was done to devitalize the spores and at the same time, if possible, to prevent heating sufficiently high to destroy any

EXPLANATORY LEGEND FOR PLATE 4

Effects of viable and devitalized spores of *Ustilago nuda* on seedlings of Han River barley sown in flats in the greenhouse Feb. 25 and Mar. 8, 1924. Seed all dehulled except those producing plants shown in H.
A.—Inoculated, sown ¾ of an inch deep; 68 per cent emergence.
B.—Uninoculated, sown ¾ of an inch deep; 96 per cent emergence.
C.—Inoculated, sown 1½ inches deep; 16 per cent emergence.
D.—Uninoculated, sown 1½ inches deep; 96 per cent emergence.
E.—Uninoculated, sown 1½ inches deep; 86 per cent emergence.
F.—Inoculated with devitalized spores; sown 1½ inches deep; 94 per cent emergence.
G.—Inoculated with viable spores; sown 1½ inches deep; 48 per cent emergence.
H.—Hulled; inoculated with devitalized spores; sown 1½ inches deep; 98 per cent emergence.



Infection of Barley by Ustilago nuda

Plate 4

(For explanatory legend see p. 272)

toxine that the spores might contain. The spores were then filtered from the solution and left on the filter to dry. A germination test proved these spores to be dead. The untreated spores germinated more than 80 per cent.

Five dehulled seeds of Han River barley inoculated with the devitalized spores and sown on moist sterile filter paper in a Petri dish showed no signs of abnormality while some of the seedlings from seed similarly treated but inoculated with viable spores showed some of the abnormal symptoms observed in seedlings from seed inoculated with viable spores and sown in the soil. The abnormal seedlings were found through microscopic examination to be heavily infected by *Ustilago nuda*.

On March 8, 1924, duplicate lots of hulled and dehulled seed of Han River and Nakano Wase barleys from the crop of 1921 were inoculated according to the regular method, one lot of each with devitalized spores and the second lot with viable spores of *Ustilago nuda*. A third lot was left uninoculated. All lots of the seed were sown 1½ inches deep in flats in the greenhouse. This seed was known to have a slight amount of natural infestation (Table V), but not sufficient to interfere seriously with the results as is shown by the controls in Table V. Seed of Red Wave wheat, one lot inoculated with devitalized and another with viable spores of *U. nuda*, and of an uninoculated control, was sown in similar flats to determine the effect of these spores on wheat seedlings. If the injury of the barley seedlings were due to a toxin produced on the surface of the seed by either the germinated or ungerminated spores, wheat seedlings might also be injured by inoculating the seed with these spores of *U. nuda*.

The emergence of the barley seedlings from dehulled seeds inoculated with viable spores was poor (Pl. 4, G, and Pl. 8, E), as had been previously noted with that type of inoculation. The spores killed by heat had no visible effect on the seedlings (Pl. 4, F and H, and Pl. 8, D and F). The stands were as good as those of the control (Pl. 4, E, and Pl. 8, B, C, and H). A high percentage of loose-smut developed in Han River plants from the dehulled seed which had been inoculated with viable spores (Pl. 7, C). More smut developed in plants from hulled seed of Han River inoculated with viable spores than occurred in corresponding plants from uninoculated seed. Plants from seed inoculated with devitalized spores were smut-free (Pl. 7, D). None of the wheat seedlings showed any signs of injury regardless of how the seed was treated. The emergence was equally good in each case and the plants were normal at maturity. (Table V.)

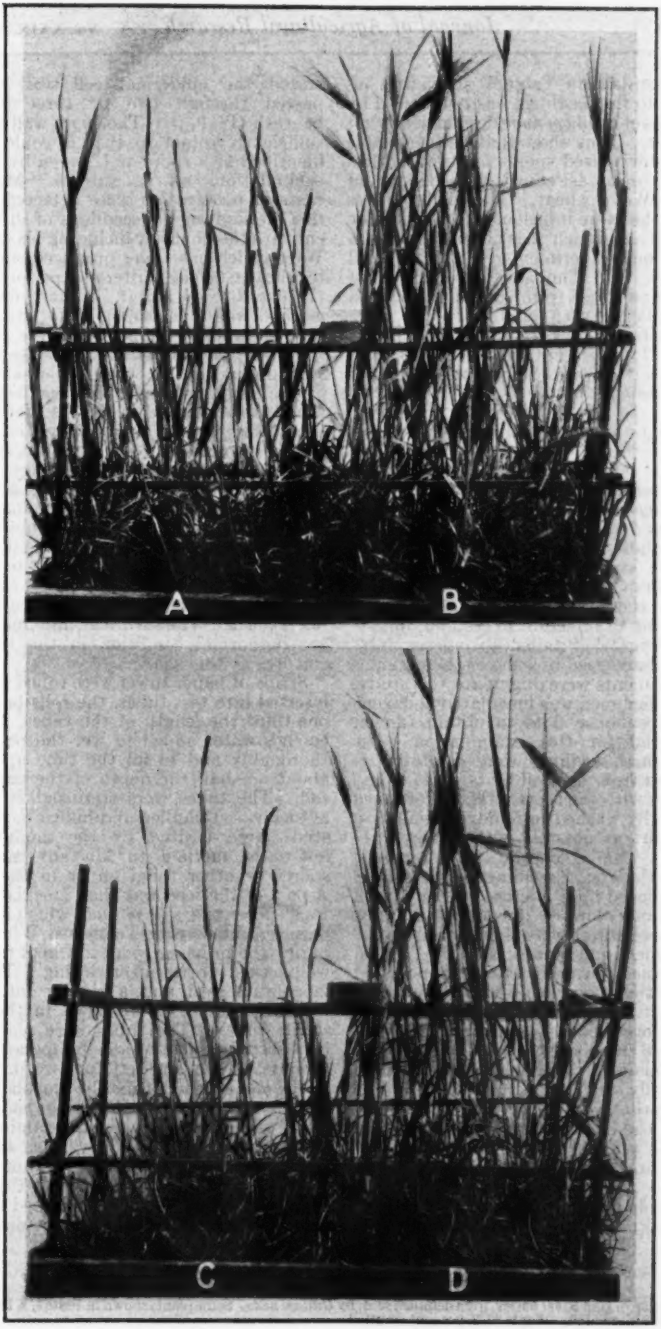
TABLE V.—*Effects of viable and devitalized spores of Ustilago nuda on barley and wheat seedlings from seed sown 1½ inches deep in the greenhouse*

Variety	Treatment previous to inoculation	Type of spores used	Number of plants from 50 seeds	Per cent of emergence	Number of smutted plants	Per cent of smut
Han River	Dehulled	None	43	86	1	2.33
Do	do	Devitalized	47	94	2	4.26
Do	do	Viable	24	48	24	100.00
Do	Hulled	None	49	98	3	6.12
Do	do	Devitalized	44	88	0	0
Do	do	Viable	48	96	5	10.42
Nakano Wase	Dehulled	None	43	86	0	0
Do	do	Devitalized	41	82	0	0
Do	do	Viable	16	32	0	0
Do	Hulled	None	46	92	0	0
Do	do	Devitalized	45	90	0	0
Do	do	Viable	46	92	0	0
Red Wave (wheat)	No treatment	None	48	96	0	0
Do	do	Devitalized	49	98	0	0
Do	do	Viable	49	98	0	0

EXPLANATORY LEGEND FOR PLATE 5

Infection of Tennessee Winter barley from dehulled seed by *Ustilago nuda*. Same plants shown in Plate 3, A to D.

A.—Seed inoculated. Plants 78.57 per cent smutted.
B.—Seed uninoculated. No smut.
C.—Seed inoculated. Plants 100 per cent smutted.
D.—Seed uninoculated. No smut.



Infection of Barley by Ustilago nuda

Plate 5

(For explanatory legend see p. 274)

The data in Table V show that injury to the seedlings was not caused by spores of *Ustilago nuda* devitalized in hot water. They show that neither viable nor devitalized spores had any noticeably injurious effect on seedlings of Red Wave wheat. The viable spores had the same injurious effect on barley seedlings which has been shown in previous experiments (Pl. 4, G and Pl. 8, E). The surviving plants of Nakano Wase from seed dehulled and inoculated with viable spores were smut-free, while those of Han River were 100 per cent smutted (Pl. 7, C). This agrees with the results of previous experiments.

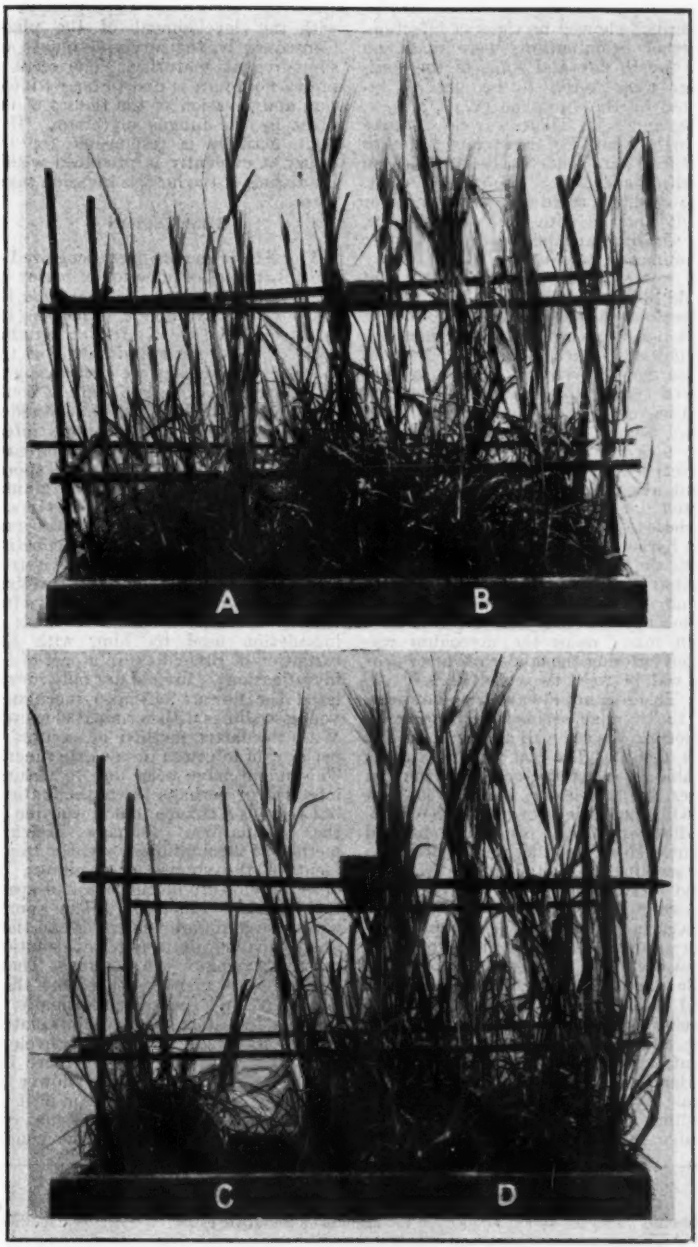
CYTOLOGIC EVIDENCE OF SEEDLING INFECTION

After noting the injurious effects of *Ustilago nuda* on the seedlings of barley, it was decided to make in addition to the studies of possible toxicity, a microscopic study of seedling infection to obtain actual proof of infection and to learn the possible relationship of the amount of infection to seedling injury. Seedlings that failed to emerge from inoculated seed in some of the previous experiments were dug up for this study. Dehulled seed was inoculated and sown in greenhouse flats to obtain further material for this examination. The abnormal seedlings were carefully removed from the soil by taking a clump of dirt with the roots. They were then carefully washed to remove as much of the dirt as possible without any rubbing. The coleoptile was removed from the young seedling with a scalpel and placed on a microscopic slide with the inner surface downward. A few drops of water were then placed on the specimen and pains taken to spread the coleoptile so that it did not fold when the cover glass was gently pressed over it. Glycerin was then added, a drop occasionally, and the slides remained in good condition for study for several days. These slides were studied carefully under the microscope. The coleoptile is sufficiently translucent so that the cellular structure is easily visible with the proper manipulation of the microscope. After considerable search a germinated spore was found adhering to the epidermis of the coleoptile, and the mycelium had penetrated the epidermal cell wall and passed through two or three cells beneath (Pl. 9, *j*). The spore wall was sufficiently intact so that it could be identified as a spore of *U. nuda* by the echinulations on its surface. Many cases of penetration were detected by this method on the seedlings of different varieties of barley including Nakano Wase which has never produced smutted heads in the writers' experiments (Pl. 9, *c*, *d*, *f*, *i*, *j*, and *l*). A more direct method was then employed for studying penetration, one in which the possibility of contamination was reduced to a minimum and in which no washing of the seedlings was necessary before the examination was made. A method similar to the one employed by Tisdale (*18*) in his studies of the penetration of the root hairs of flax seedlings by *Fusarium lini* was used. As the smuts infect the seedlings of small grains so early in their development, this method was considered satisfactory. The seedling is not far enough advanced to be drawing much, if any, of its food from the soil at the time when infection takes place. The seedlings apparently are in normal condition in the tube cultures at this age.

Strips of paper towel were rolled and inserted into test tubes, the rolls being one-third the length of the tubes, and enough water added to wet the paper thoroughly and to fill the tube up to about one-half the depth of the paper roll. The tubes were sterilized in an autoclave. Dehulled and hulled barley seeds were treated by the modified hot-water method to kill any smut spores or other fungi on or in them. A part of the seed was then inoculated with spores of *Ustilago nuda* which had been collected from Tennessee Winter barley the previous year and kept in a well-corked bottle after drying. This inoculated seed and a similar lot of uninoculated seed were sown in these sterile tubes. The tubes were placed in a dark closet at room temperature (about 21° C.) and left for two days, at the end of which time the inoculated seeds which were germinating had a frosty appearance. When examined under the microscope this whitish substance proved to be the mycelium of germinated spores of *U. nuda*. Coleoptiles of these seedlings examined according to the method previously

EXPLANATORY LEGEND FOR PLATE 6

Infection of Han River barley, from dehulled seed, by *Ustilago nuda*. Same plants shown in Plate 4, A to D.
A.—Seed inoculated. Plants 82.34 per cent smutted.
B.—Seed uninoculated. No smut.
C.—Seed inoculated. Plants 100 per cent smutted.
D.—Seed uninoculated. No smut.



Infection of Barley by Ustilago nuda

Plate 6

(For explanatory legend see p. 276)

described showed no signs of infection. Further examinations were made on the fourth day and some of the seedlings were found to be heavily infected by these hyphae (Pl. 9, *a*, *b*, *e*, *g*, *k*, and *m*). There was no mistake as to the identity of the fungus. Similar cultures were made in Petri dishes on sterile paper with the same result. The seedlings were easy to remove for study and, as with the tube cultures, no washing was necessary before the examination was made. In this way the spores were not disturbed and could be found attached to the hyphae. Infections of seedlings of the following varieties were found by this method: Tennessee Winter, Han River, Nakano Wase, and Smyrna (C. I. 195), a two-rowed barley. In one case the first two leaves of a seedling of Han River were found to be heavily infected (Pl. 9, *h* and *n*). Nakano Wase, the variety which has not shown any smut at maturity, apparently was infected as easily as the susceptible varieties (Pl. 9, *k* and *m*). One hull-less barley, C. I. 2222 was sown in the dish cultures and examined to a limited extent but no infections were found. Reports indicate that the naked barleys smut heavily in nature.

In many cases the mycelium was found entering the epidermis at or near the wall between two cells (Pl. 9, *b*, *e*, *g*, *j*). This was not always true, however, as the hyphae seemed to be able to penetrate the cell wall at any point (Pl. 9, *a*, *d*, *f*, *i*). The fact that the fungus is able to infect the coleoptile and, in cases, the first leaves, is not proof that it will reach the growing point of the seedling and finally result in a smutted plant. However, the injury caused to the seedling (Pl. 3, 4, 7, and 8) is a very good indication that its activities are not confined to the coleoptile and the superficial tissues of the plant. Furthermore, the high percentages of loose-smut occurring in the plants of susceptible varieties grown from inoculated seed is definite proof that the fungus reaches the growing point of the seedling and keeps pace with its development. With Nakano Wase the fungus evidently penetrates beyond the superficial layers, as evidenced by severe seedling injury (Pl. 3 and 8) but is unable for some reason to keep pace with the development of the plant. Consequently, the surviving plants are smut-free at maturity. The seedling injury no doubt is due to heavy infection and invasion of the tissues of the plant by the fungus mycelium. If a toxin actually is responsible for the injury it evidently is produced within the tissues of the host as invasion takes place.

DISCUSSION

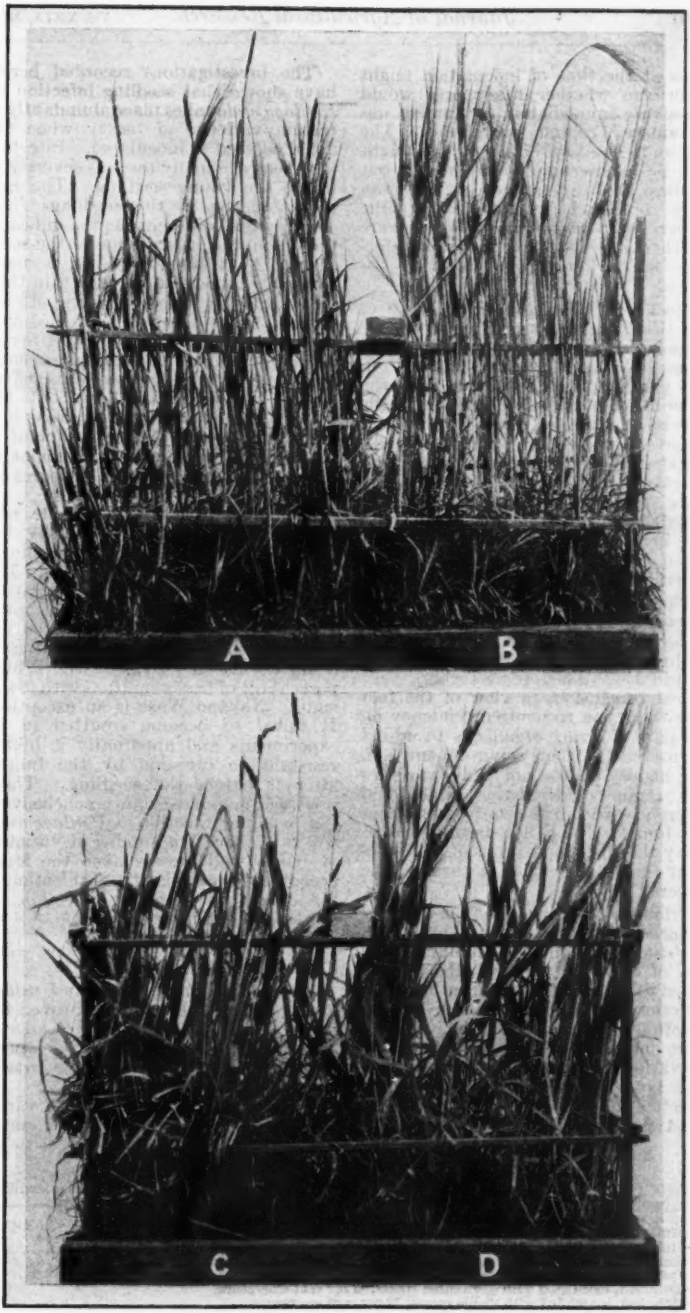
Floral infection has been recognized as the only means of infection of barley by the loose-smut fungus, *Ustilago nuda*, for many years. No doubt, if Jensen (*12*) had continued his infection studies with dehulled seed and included the loose-smut fungus after discovering it he would have found that it could infect the seedlings of barley. Maddox (*16*) through whose work floral infection was first established, apparently overlooked the possibility of the spores remaining viable between the glumes and the kernel when the flower is inoculated. He failed to consider the fact that the hulls furnish a very effective obstruction to infection. This is true for both barley smuts. Since the time of Maddox other investigators have followed practically the same methods of inoculation used by him, with the exception of Brefeld (*3*) in a part of his investigations. Brefeld not only inoculated the flowers but also inoculated young seedlings with germinated spores. With the latter method he secured 1 per cent of infection in one experiment. This might have been due to natural infection or perhaps to contamination, but there is a chance that it was due to the inoculation. Perhaps Brefeld's method of inoculation or else the treatment of the spores, or seedlings, or both, previous to inoculation had upset their normal functions. The spores were first germinated in water and then finely divided in a nutrient solution before spraying on the seedlings. There no doubt are many reasons why they might not have functioned normally.

Other investigators have inoculated the flowers at various stages of development and have found that more infection takes place if the flower is inoculated in one stage than if it is inoculated in another. It seems entirely possible that the weather condi-

EXPLANATORY LEGEND FOR PLATE 7

Effects of viable and devitalized spores of *Ustilago nuda* on Han River barley. C and D were shown in Plate 4.

A.—Seed dehulled and inoculated. Plants 91.89 per cent smutted.
B.—Seed dehulled and uninoculated. No smut.
C.—Seed dehulled and inoculated. Plants 100 per cent smutted.
D.—Seed hulled and inoculated with devitalized spores. No smut.



Infection of Barley by Ustilago nuda

Plate 7

(For explanatory legend see p. 278)

tions at the time of inoculation might determine whether the spores would germinate immediately, or remain ungerminated behind the glumes. The age and condition of the spores might also be a determining factor. In our studies low percentages of infection were obtained by inoculating the flowers, and the stage the flowers were in when inoculated made little difference in the amount of infection obtained.

Hecke (*9*) illustrates a mycelium in the tissues of the scutellum of barley kernels. It does not seem entirely impossible that Hecke was mistaken in the identity of the fungus. On the other hand, it is possible that the fungus infects at flowering time to a certain extent. Certain local climatic conditions might be more conducive to floral infection. Should floral infection take place there still remains the possibility that the internal mycelium would not become active when the seed is sown. In this respect the control by superficial disinfection is significant.

There may be specialized strains or races of the fungus *Ustilago nuda* such as have been found in other fungi, including some of the cereal smuts (*6* and *17*). This seems to be a very logical conclusion, in view of the fact that there is a recognized tendency on the part of living organisms to adjust themselves to their environment. In the higher plants not only varieties and strains but distinct individual differences within the variety or strain are found. It is not beyond possibility that there are strains of the loose-smut fungus which infect through the flower.

Brioli (*4*), working with four varieties of barley, says that he found mycelium of *Ustilago nuda* in the scutellum of the seed of only one variety. He was inclined to the opinion that the other three varieties were resistant. The lack of infection might have been more nearly what happens in nature. However, the varietal differences of the host plant may be a determining factor. Some varieties may be more susceptible to this type of infection.

The investigations recorded herein have shown that seedling infection by *Ustilago nuda* takes place abundantly in certain varieties of barley when the dehulled seed is inoculated. Infection is so heavy in many cases as severely to injure the young seedling. The rate of emergence of the seedlings from seed sown at different depths indicates the amount of injury done to the seedlings. An injured seedling is more capable of emerging through thin than it is through thicker layers of soil within the depths at which sowing is customary. Loose soils are more favorable to emergence than are close, compact soils. There may be a toxic effect of the fungus on the host but the data indicate that if a toxin exists it is produced internally after the plant is infected. There are no indications of injury due to the presence of spores on the seed.

Seedlings of both susceptible and resistant barleys are injured by the fungus. Infection studies have shown that both types become infected. The germ tube is capable of penetrating directly the cell walls of the coleoptile and at least the first two leaves of the young seedlings. The surviving plants from inoculated seed produced a high percentage of heads smutted with loose-smut. Nakano Wase is an exception. It failed to become smutted in all experiments and apparently is highly resistant to invasion by the fungus after it enters the seedling. These investigations have shown conclusively that seedling infection by *Ustilago nuda* will take place in a number of varieties of barley and have shaken the long-accepted theory that floral infection is the only type of barley infection by the loose-smut fungus, *U. nuda*.

SUMMARY

Floral infection of barley by *Ustilago nuda* has been accepted as proved for many years.

Surface disinfectants have been found to control loose-smut in certain varieties of barleys.

Dehulled seed of a number of varieties of barleys inoculated with spores

EXPLANATORY LEGEND FOR PLATE 8

Effects of viable and devitalized spores of *Ustilago nuda* on Han River and Nakano Wase barley seedlings grown from hulled and dehulled seed sown 1½ inches deep in flats in greenhouse, on Mar. 8, 1924.

Han River:

A.—Hulled, inoculated with viable spores; 96 per cent emergence.

B.—Hulled, uninoculated; 93 per cent emergence.

Nakano Wase:

C.—Dehulled, uninoculated; 86 per cent emergence.

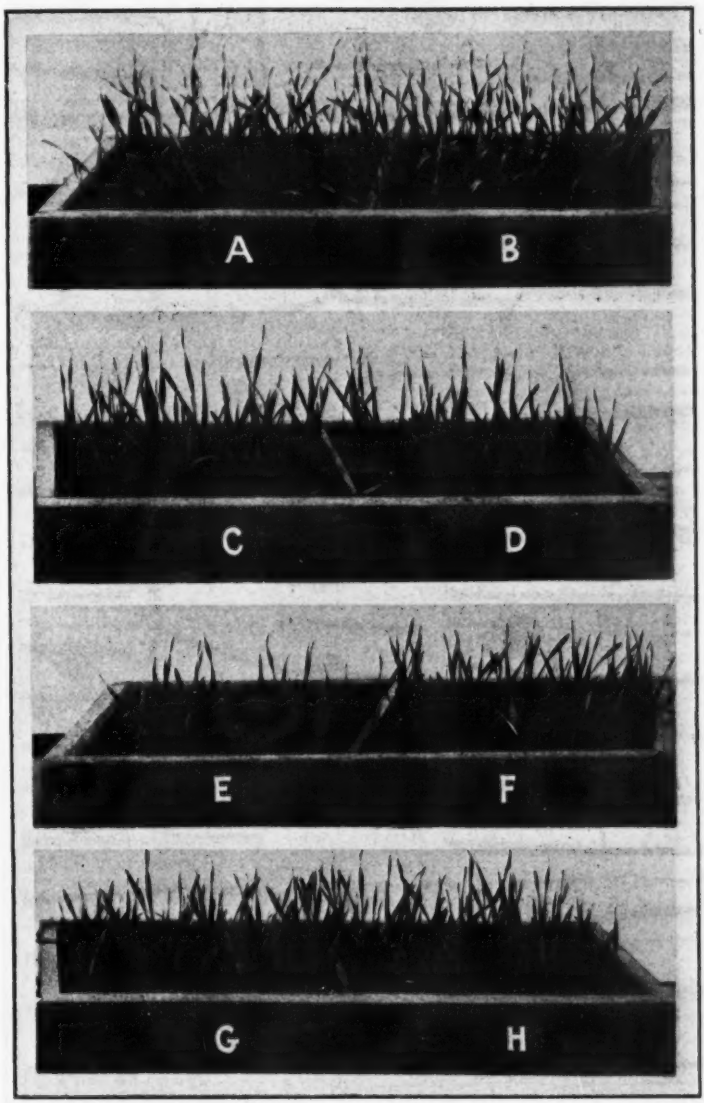
D.—Dehulled, inoculated with devitalized spores; 82 per cent emergence.

E.—Dehulled, inoculated with viable spores; 32 per cent emergence.

F.—Hulled, inoculated with devitalized spores; 90 per cent emergence.

G.—Hulled, inoculated with viable spores; 92 per cent emergence.

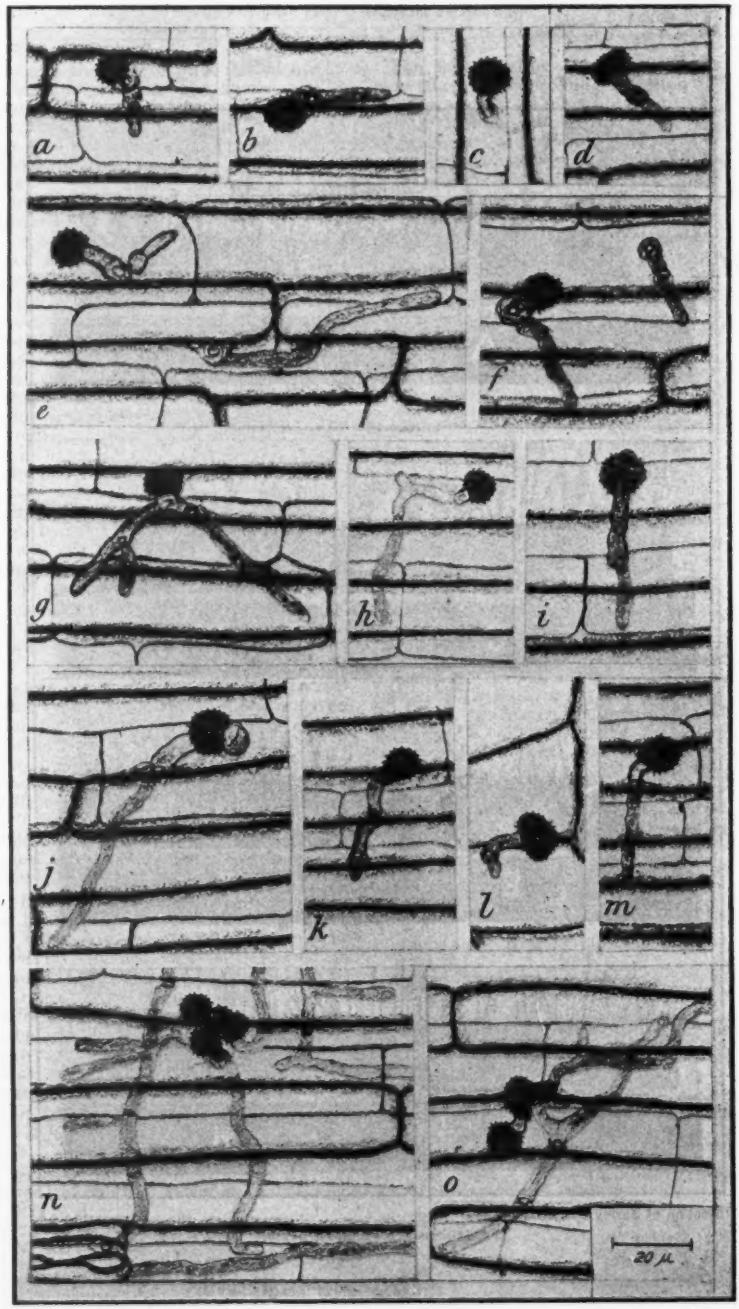
H.—Hulled, uninoculated; 92 per cent emergence.



Infection of Barley by Ustilago nuda

Plate 8

(For explanatory legend see p. 280)



Infection of Barley by Ustilago nuda

Plate 9

(For explanatory legend see p 283)

of *Ustilago nuda* produced plants with a high percentage of loose-smut.

Nakano Wase barley remained smut-free even though the seed was dehulled and smutted.

Seedlings from dehulled-inoculated seed of all varieties of barley studied were severely injured, and many of them failed to emerge.

Seedlings from dehulled-inoculated seed sown three-fourths of an inch deep emerged better than those from similar seed sown 1½ inches deep.

Seedlings from hulled-inoculated seed were not noticeably injured.

Devitalized spores of *Ustilago nuda* were not harmful to barley seedlings grown from the inoculated seed.

Neither viable nor devitalized spores of *Ustilago nuda* were harmful to seedlings of Red Wave wheat grown from inoculated seed.

A microscopic study revealed infection of the coleoptile and first leaves of the plumule of barley seedlings by *Ustilago nuda*. Both susceptible and resistant varieties were infected.

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EXPLANATORY LEGEND FOR PLATE 9

Infection of barley seedlings by *Ustilago nuda*.
Coleoptile infection in sterile tube and dish culture: *a*, Smyrna (C. I. 195), a two-rowed barley; *b*, *e*, and *g*. Nakano Wase; *k* and *m*, Tennessee Winter; *o*, Han River.
Coleoptile infection in soil: *c*, *d*, *f*, and *i*, Han River; *j*, Tennessee Winter. Infection at base of coleoptile, in soil; *l*, Han River.
Infection of first leaf in sterile dish culture: *h* and *n*, Han River.

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THE EFFECT OF FEEDING THYROID ON THE PLUMAGE OF THE FOWL [1]

By L. J. COLE, *Chairman, Department of Genetics, University of Wisconsin,* and D. H. REID, *Head Professor of Poultry Husbandry, Agricultural and Mechanical College of Texas*

The assumption of male plumage by the female fowl has frequently been observed and commented upon. When it occurs in nature it is usually found associated with a disturbance of the ovary (*1*),[2] and it may be produced experimentally as a sequel of ovariotomy (*3*). The writers are not aware that the spontaneous assumption of female plumage by the male fowl has been reported (leaving out of consideration those breeds, such as the Sebright Bantam, in which it is a normal inherited characteristic), though it is a process which occurs annually in many dimorphically colored birds, such as the scarlet tanager, bobolink, and indigo bunting. Goodale (*4*, *5*) has succeeded, however, in inducing henfeathering in males by a combination of castration and implantation of ovarian tissue. More recently, Torrey and Horning (*6*) have reported that when dried thyroid was fed to normal growing male chicks they developed female plumage instead of that natural to their sex. No change of plumage was effected, however, by feeding thyroid to normal females nor to castrated males or females. These results are of interest and seem important, even though a trial reported by Crew and Huxley (*2*) has failed to confirm them. It would seem that for the present the accumulation of additional evidence is more to be desired than extended discussion of the possible action of glandular secretions on the development and expression of secondary sexual characters.

A preliminary trial of the effects of feeding thyroid to adult males was made by the writers at the Wisconsin Agricultural Experiment Station in the late winter and early spring of 1923. Adult males were used for the test, partly because no chicks were available at the time and partly to see whether or not any effect would be visible in the replaced feathers of the grown bird.

Ten Brown Leghorn cockerels nearly a year of age and one old male were used in the experiment. All had normal plumage of the breed with respect both to color and form of feathers. They were placed in individual coops (about the size of exhibition coops) in the poultry building, but were divided into four lots with respect to treatment, as follows:

In addition to the regular ration—

LOT 1, consisting of four birds, received every other day at first and later, daily, 400 mgm. of desiccated thyroids[3] containing 0.2 per cent iodine, or 0.8 mgm. iodine per dose. Torrey and Horning started with 50 mgm. of thyroids when their chicks were four weeks of age and increased the dose from time to time to 330 mgm. at the end of 15 weeks. They do not state the weights of their birds, but the writers have assumed that these weighed about 4 pounds when 19 weeks of age, and the dose for the birds in the present experiment, which averaged about 4.75 pounds, was calculated at approximately the same proportion in relation to weight. The material was easily administered in capsules.

LOT 2, two birds, each fed 1.05 mgm. KI, in capsules, the iodine content being equivalent to that in the thyroids. These were fed at the same intervals as the thyroids.

LOT 3, two birds, each given at the same periods by pipette 1 cc. of 0.08 per cent solution of iodine in 28.5 per cent alcohol.

LOT 4, three birds, controls, received no dosage.

The experiment was begun on February 9, all birds being given their doses on succeeding alternate days up to March 1; after that they were dosed daily until the experiment was con-

[1] Received for publication Apr. 8, 1924. Issued January, 1925. Paper No. 42, from the Departments of Genetics and Poultry Husbandry, Wisconsin Agricultural Experiment Station. Published with the approval of the Director.

[2] Reference is made by number (italic) to "Literature cited," p. 287.

[3] The writers desire to express their appreciation to Armour & Co., Chicago, for their kindness in furnishing the thyroids used in this experiment.

cluded on May 2. On February 20, a patch of feathers was pulled from the cape and wing bow of each bird and saved for future reference. (See Pl. 1.) On March 1, a patch from the saddle and one sickle feather were also pulled. All birds were weighed weekly and notes were kept on general health and on growth of new feathers. Such results as were obtained may be stated briefly.

There was considerable variability in the rate at which new feathers replaced those which were pulled, depending in part, apparently, on the state of health of the individual bird, but also, as will be noted later, on the medication. In the better-developed cases the new feathers were of full length by May 2, but in the others comparatively little replacement had occurred. In no case was the sickle replaced.

It will simplify matters to make the summary statement that in all lots except that receiving thyroids the new feathers were in all respects normal both as to shape and coloration. This is doubtless what was to be expected, since experiments on mammals have shown little effect caused by administering iodine, free or in inorganic combination, except in cases of iodine deficiency or of subnormal activity of the thyroid gland. There is no reason to suppose that either of these conditions existed in the present instance.

The new feathers on the thyroid-fed birds were, however, strikingly different from those which were pulled, as is indicated in the accompanying tabulation. There was considerable variation, as will be noted, and it can not be said that the replaced feathers were characteristically female. As relates to color, there was an evident action toward the reduction of red pigment, varying in degree in the different birds, but tending to be arranged in "stippling" when present. This was particularly true in the new cape feathers of bird No. 441, which were decidedly femalelike in appearance. In all cases the red was broken and much more irregular in distribution than in normal male feathers. The resemblance to female feathers was much more striking in respect to shape and structure, the broad, rounded ends contrasting strongly with the pointed tips of the male plumage on these parts. Furthermore, the great reduction or absence of the zone of "free" barbs, that is, of barbs lacking barbules and hooks, so characteristic of the normal male feathers, was very evident, and constitutes another resemblance to female plumage.

Summing up, it may be said that the feeding of desiccated thyroids to male Brown Leghorn fowls in certain amounts profoundly modifies feathers grown while the material is being administered. The feathers produced under these conditions are not typically female in type, but they do show distinct female characteristics. There would seem to be in this case no question of altering the sex of the bird, nor in all probability of the sex hormones. Nor is it likely that the administered thyroid material acts through the mediation of the thyroid gland of the bird. It seems altogether more probable that the thyroid fed acts directly in influencing the metabolism of the developing feather germ. The very evident tendency to an increased melanism may indicate a higher oxidation of the pigment products; the explanation of the change in form must be less direct.

The feeding of iodine, free and in inorganic combination, gave no result comparable to that obtained from dessicated thyroids containing an equivalent amount of iodine.

One other apparent effect of the thyroid feeding deserves mention, namely, that the replacement of feathers on the birds receiving thyroids was noticeably more rapid than on the others. Since some of the birds were suffering from "colds" and were not in the best of health during the trial, however, it would not be safe to make too definite a pronouncement on this point. If the feeding of thyroid should prove to have a stimulating effect on feather development it might prove useful in inducing quick and uniform molting, a factor of considerable practical importance. It is proposed to test this further by later experiments.

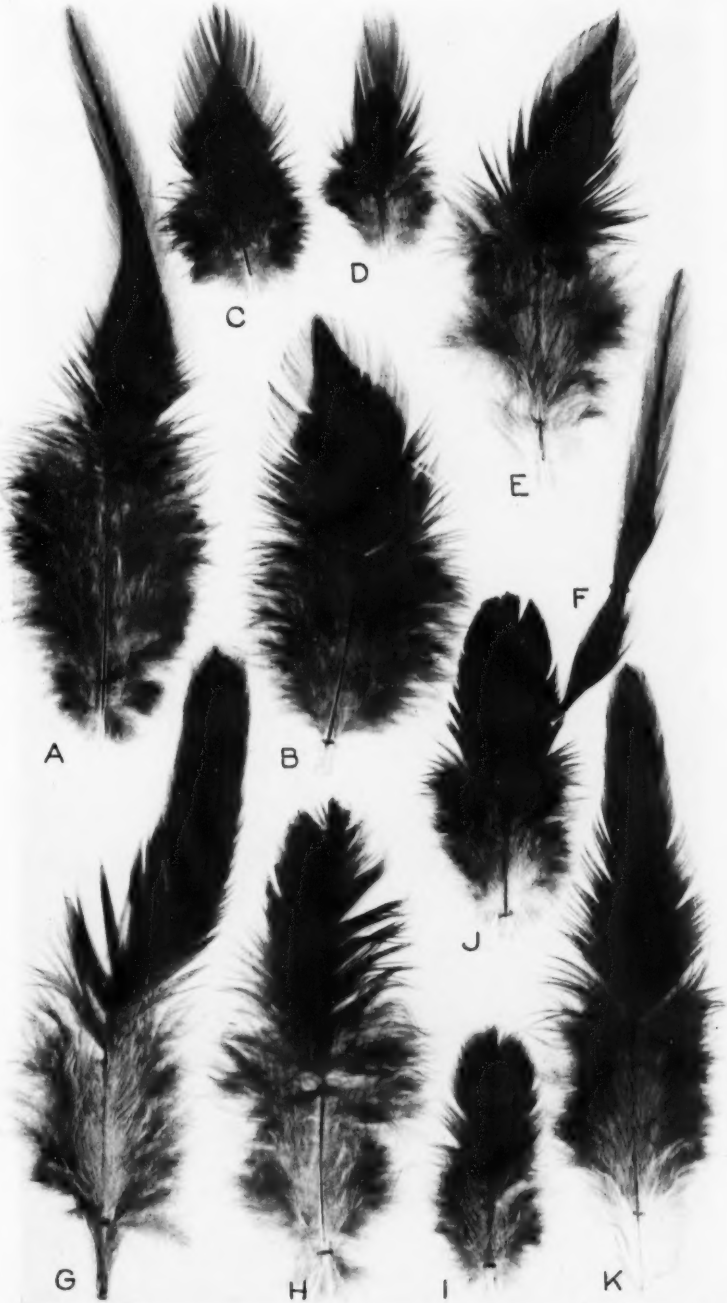
EXPLANATORY LEGEND FOR PLATE 1

A, B, and C.—Normal feathers from saddle, cape, and wing bow, respectively, of bird No. 448. Cape and wing bow feathers pulled February 20; saddle, March 1.

D, E, and F.—New feathers grown by bird No. 448, replacing those pulled (D, wing bow; E, cape; F, saddle). The new feathers, which were pulled on May 2, appear normal in shape, structure, and coloration, apparently not having been affected by the free iodine in alcoholic solution administered to this subject.

G, H, and I.—New feathers (May 2) from saddle, cape, and wing bow, respectively, from bird No. 441. These feathers show modification of shape, structure, and coloration, approaching the female type in these respects. The stippling in the cape feather is closely similar to that which occurs in females. These modifications are attributed to the desiccated thyroids fed.

J and K.—New cape and saddle feathers from bird No. 444, following feeding of desiccated thyroids. The melanistic effect was especially pronounced in this bird.


A
B
C
D
E
F
G
H
I
J
K

COMPARISON OF NEW FEATHERS IN THYROID-FED BIRDS WITH THOSE OF NORMAL BIRDS

	Cape	Wing Bow	Saddle
Normal birds.	Exposed portion generally red, base black; red may occupy whole distal half of feather or be reduced to a border. Distinctly pointed; with broad zone of free barbs.	Similar to cape but occasionally with black spot at tip. Same general shape, but shorter.	Varying from almost wholly red to red with black middle stripe. Long (some 12 cm.); narrowly attenuated distally; pointed. Wide zone of free barbs.
Bird No. 441..	Black, with *definite red stippling* near tip. Broadly rounded, without zone of free barbs.	Black with a considerable quantity of red more or less irregularly intermixed; tendency to stippling. Broadly rounded, without "free zone."	Black with considerable red irregularly distributed. Elongate (11 cm.) but broad, and rounded at tip; very little "free zone."
Bird No. 440..	Black; little if any red. Broadly rounded; without "free zone."	Black. Broadly rounded; without "free zone."	Dull black with some irregular red; tendency to stippling. Broad, rounded, without "free zone"; length 8 cm.
Bird No. 444..	Mostly black; a few feathers with a small amount of red on margin. Bluntly rounded; without "free zone."	Black with small amount of red, showing tendency to stippling. Bluntly rounded; no "free zone."	Black (no red). Elongate (11 cm.); with somewhat the taper of male saddle feathers but broader and rounded at tip; narrow "free zone" near tip.
Old male.....	Black or black with little red. Broadly rounded; without "free zone."	None available. (This bird pulled the new feathers he could reach as they grew.)	None available.

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POLYSCELIS MODESTUS GAHAN, A MINOR PARASITE OF THE HESSIAN FLY [1]

By P. R. MYERS [2]

Assistant Entomologist, Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION AND HISTORY

The hymenopterous parasite which is the subject of this paper was first reared from the Hessian fly (*Phytophaga destructor* Say) in the summer of 1915 by the late W. R. McConnell and the author at the Hagerstown, Md., Laboratory of the Cereal and Forage Insect Investigations of the Bureau of Entomology, United States Department of Agriculture, when a heavy infestation of that insect pest of wheat occurred generally throughout the wheat-growing region of the Eastern States.

At that time specimens of this species were submitted for determination to A. B. Gahan, of the Bureau of Entomology, who tentatively determined them as *Polyscelis* sp. A subsequent examination of the material and literature, however, revealed it to be a new species and accordingly it was described by Mr. Gahan [3] in 1922 under the name *Polyscelis modestus*.[4]

Although knowledge of this species is rather meager, it seems advisable to record the data which have accumulated concerning its life history, especially in view of the fact that the species is uncommon and no recoveries of it have been made since 1918.

DISTRIBUTION

The first specimens of this species were reared from Hessian fly puparia collected by the author in 1915 near Hanover, Pa. Later on, however, specimens were reared from material collected in the following five additional localities in the States of Maryland and Pennsylvania: Hagerstown, Md. (W. R. McConnell, E. M. Craighead); Andersonburg, Carlisle, Gettysburg, and Perkasie, Pa. (P. R. Myers).

HOSTS

Normally *Polyscelis modestus* is a primary, solitary parasite of the Hessian fly. The primary nature of its attack on this host has been demonstrated on several occasions by breeding it experimentally in the laboratory upon nonparasitized Hessian fly puparia. It attacks the host usually in the larval stage but cases have been found where it has developed also upon Hessian fly pupae. It feeds externally on its host within the puparium. Completing its development within the puparium, it emerges therefrom as an adult.

This species has also been shown to be a secondary parasite by the rearing of a single minute male from a puparium which, when opened, was found to contain the cocoons of a platygasterid parasite, probably *Platygaster vernalis* (Myers).

In another instance two eggs of *P. modestus* were deposited within the same puparium. Shortly after the hatching of these eggs one of the resulting larvae attacked the other and by the following day had consumed its contents. Evidence was also found indicating that young larvae probably attack and destroy the eggs of their own species.

These cases of hyperparasitism and hyperpredacity plainly indicate that upon occasion this species can develop upon parasitic larvae, and probably pupae, of its own or some other species as well as on the larvae and pupae of the Hessian fly.

ECONOMIC IMPORTANCE

Although this species was easily reared upon nonparasitized Hessian

[1] Received for publication July 26, 1924. Issued January, 1925.

[2] The biological data contained in this article were obtained principally from experiments conducted by the late W. R. McConnell in the laboratory of Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture, at Carlisle, Pa., during the summer of 1917. The writer gladly expresses his indebtedness to Miss Esther H. Hart for the drawings of the adult and the leg, wings, and antennae of adults; and to C. C. Hill for the drawings of the egg, larva, pupa, and parts of larva, and for kindly criticisms.

[3] GAHAN, A. B., DESCRIPTIONS OF MISCELLANEOUS NEW REARED PARASITIC HYMENOPTERA. Proc. U. S. Nat. Mus., 61: 11–12. 1922.

[4] Order Hymenoptera, superfamily Chalcidoidea, family Pteromalidae.

fly puparia in the laboratory both at Hagerstown, Md., and Carlisle, Pa., yet in the course of the investigations it has been possible to secure only 13 specimens of this species from material collected in the field. In view of the fact that so few specimens have been reared from material collected in the field, it would seem that this species is one of the rare and less important parasites of the Hessian fly. Adults have been reared from Hessian fly puparia, both of the fall and spring generations. The maximum percentage of parasitism obtained from material of the spring generation was 0.85 per cent, whereas in the material of the fall generation it was 1.5 per cent.

EGG

The egg (fig. 1, *a*) is translucent whitish in color. In shape it is rather ovate with the cephalic end larger and more obtuse than the caudal end. It is about two and one-half times as long as its greatest width. The ventral region is slightly concave and the dorsal region convex. The chorion is thin and elastic and is almost entirely densely spinose. There is a small circular area at the caudal end bare of spines. The spines are of uniform length everywhere except close to the periphery of the smooth area. Here they shorten declivitously. The length of the egg varies from 0.34 to 0.38 mm. and its greatest width varies from 0.14 to 0.15 mm. The average measurement of 10 eggs was 0.36 mm. in length by 0.15 mm. in greatest width.

INCUBATION

During the month of July when these experiments were conducted, the period of incubation varied, occupying from one and one-half to two days. In hatching, the young larva pushes its head against the ventral portion of the cephalic end of the egg, stretching the elastic chorion at this point into a blunt protuberance. After pushing against the chorion for some seconds the larva withdraws into its normal position. This process is repeated after short intervals for an hour or more before hatching actually occurs. During the effort of the larva to break through the chorion the posterior part of the shell is contracted irregularly by the forward movement of the larva, and the mandibles of the young larva can be observed opening and closing within.

PRIMARY LARVA

The newly hatched larva is about 0.35 mm. long, or about the length of the egg itself. It is widest at the cephalic end but tapers gradually to a rather acute point at the caudal end. There are rather prominent tubercles on each side of the anterior body segments, each of which is surmounted by a coarse seta. The head bears a pair of antennae and some fine setae. The mouth, which is of a protruding suctorial form, is readily discernible. In its attack upon its host the young parasitic larva may attach itself to any part of the host's body.

MATURE LARVA

The mature larva (fig. 1, *b*) varies from 1.5 mm. to 2 mm. in length and from 0.5 mm. to 0.6 mm. in width. The average length of 10 larvae was 1.7 mm. and the average width was 0.54 mm. It is of a typical chalcidoid form, somewhat spindle shaped, slightly curved, tapering to both extremities but more acutely toward the caudal than the cephalic end. It is white in color, smooth, shining, and bare except for two rows of fine setae along the latero-dorsal area of the first 12 segments. These setae are arranged two to a segment, one directly above the other. The body consists of 13 segments. The anal segment is divided into a dorsal and a ventral lobe, the dorsal lobe being margined posteriorly by a row of four setae. Spiracles are present on the mesothoracic, metathoracic, and the first seven abdominal segments. The head (fig. 1, *c*) when viewed from the front is slightly concave giving it a somewhat bilobed appearance. The antennae which are very short and pale brown in color are surmounted on a fleshy tubercle. The labrum is broadly ovate, slightly curved, and sparsely and finely setose. Two setae are discernible on either side of the head, laterad of the mouth. The mandibles (fig. 1, *d*) of the mature larva are about 0.03 mm. long. They are chitinous, light brown in color, simple in form and nearly straight. The superior margin of the mandible is slightly curved while the inferior margin is nearly straight. No other appendages of the mouth parts are discernible.

The duration of the larval stage varies from 4 to 11 days. The average length of the larval period for 30 larvae was slightly more than 6 days. Table I shows the individual variation of the duration of the larval and other stages.

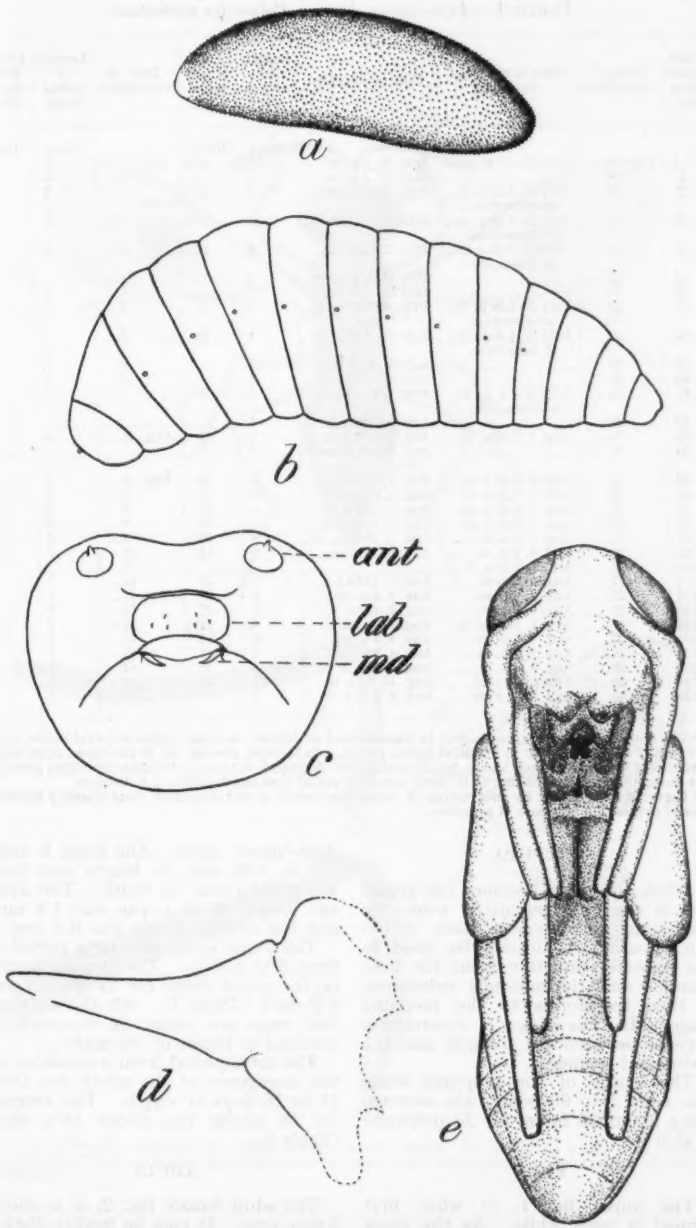

FIG. 1.—*Polyscelis modestus*: *a*, Egg, greatly enlarged (actual length 0.38 mm.); *b*, mature larva, enlarged (actual length 1.6 mm.); *c*, front view of head of mature larva, showing mouth parts much enlarged (*ant*, antenna; *lab*, labrum, *md*, mandible); *d*, mandible of mature larva, greatly enlarged (actual length 0.027 mm.); *e*, pupa, ventral view (actual length 1.8 mm.).

TABLE I.—*Life-history data on Polyscelis modestus* [a]

Host puparium No.	Date of oviposition	Date of meconial discharge	Date of pupation	Length of larval stage	Length of prepupal stage	Date of emergence	Length of pupal stage	Period from egg to adult
				Days	*Hours*		*Days*	*Days*
14	July 18	July 26, 11.45 a. m.	July 27, 2 p. m.	[b]7	26¼	Aug. 1	5	14
15	18		July 27, 2 p. m.	7		2	6	15
27	19	July 26, 3.30 p. m., at dissection.	July 27, 2 p. m.	6	22½	2	6	14
28	19	July 26, 3.30 p. m., at dissection.	July 27, 2 p. m.	6	22½	2	6	14
29	19	July 26, 3.30 p. m., at dissection.	July 27, 2 p. m.	6	22½	2	6	14
42	22		July 31, 2.30 p. m.	7				
43	22		July 31, 2.30 p. m.	7		7	7	16
47	22	July 27, 2.30 p. m., at dissection.	July 28, 9.30 a. m.	4	19	2	5	11
54	22	July 27, 2.30 p. m., at dissection.	July 28, 9.30 a. m.	4	19	2	5	11
79	24		July 31, 2.30 p. m.	5		6	6	13
80	24		July 31, 2.30 p. m.	5		6	6	13
81	24	July 31, 3 p. m., at dissection.	Aug. 1, 9.30 a. m.	6	18½	7	6	14
82	24		Aug. 1, 9.30 a. m.	6				
83	24	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	7	24	Aug. 8	6	15
84	24		July 31, at dissection.	5				
93	25	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	6	24	Aug. 8	6	14
94	25	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	6	24	8	6	14
95	25	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	6	24	8	6	14
97	25	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	6	24	8	6	14
101	26	Aug. 2, 9 a. m.	Aug. 3, 10.30 a. m.	6	25½	9	6	14
109	26	Aug. 4, 2 p. m.	Aug. 6, 10 a. m.	9	44	13	7	18
110	26	Aug. 2, 9.30 a. m.				9		14
111	27	Aug. 4, 9 a. m.	Aug. 5, 12 m.	7	27	12	7	16
116	27	Aug. 3, 6 p. m.	Aug. 4, 2 p. m.	6	20	11	7	15
123	27	Aug. 4, 9 a. m.	Aug. 5, 12 m.	7	27	13	8	17
124	27	Aug. 3, 10.30 a. m.	Aug. 4, 9 a. m.	6	22½	11	7	15
125	27	Aug. 3, 6 p. m.	Aug. 4, 9 a. m.	6	15	11	7	15
132	28	Aug. 3, 6 p. m.	Aug. 4, 9 a. m.	5	15	11	7	14
137	28	Aug. 4, 9 a. m.	Aug. 4, 2 p. m.	5	5	12	8	15
138	28	Aug. 6, 10 a. m.	Aug. 10, 10 a. m.	11	96	15	5	18
149	30	Aug. 6, 10 a. m.	Aug. 6, 5.30 p. m.	5	7½	13	7	14

[a] The data in the foregoing table may be summarized as follows: Average length of larval period for 30 larvae, 6.17 days; variation in length of larval period, 4 to 11 days; average for 23 prepupae, 24.99 hours; variation of prepupal period, 5 to 96 hours; average for 27 pupae, 6.3 days; variation for pupal period, 5 to 8 days; average for 28 adults, 14.46 days; variation period from egg to adult, 11 to 18 days.

[b] Larval stage obtained by subtracting an incubation period of two days from time elapsing between date of oviposition and date of pupation.

PREPUPA

When the larva becomes full grown and is ready to pupate it voids the meconium and reverses itself within the puparium. Turning its head in the opposite direction from the host remains and the meconial substance, it then transforms to the prepupal stage. In this stage a constriction appears between the thoracic and the abdominal segments.

The length of the prepupal stage was from 5 to 96 hours, the average being about 25 hours for 23 prepupae (Table I).

PUPA

The pupa (fig. 1, *e*) when first formed is pure white. As the pupa ages the eyes become red and the mandibles brown. After this the whole pupa very rapidly assumes a light-brown color. The pupa is from 1.5 to 1.95 mm. in length and from 0.5 to 0.65 mm. in width. The average length of 10 pupae was 1.8 mm. and the average width was 0.6 mm.

The pupal stage lasts for a period of from 5 to 8 days. The average length of the pupal stage for 27 pupae was 6.3 days (Table I). All the data on this stage are based on observations confined to pupae of the male sex.

The total period from oviposition to the emergence of the adult was from 11 to 18 days in length. The average for 28 adults was about 14½ days (Table I).

ADULT

The adult female (fig. 2, *a*) is about 2 mm. long. It may be readily distinguished from the other Hessian fly parasites by a large, faint, subcircular, fuscous spot in the middle of the fore-

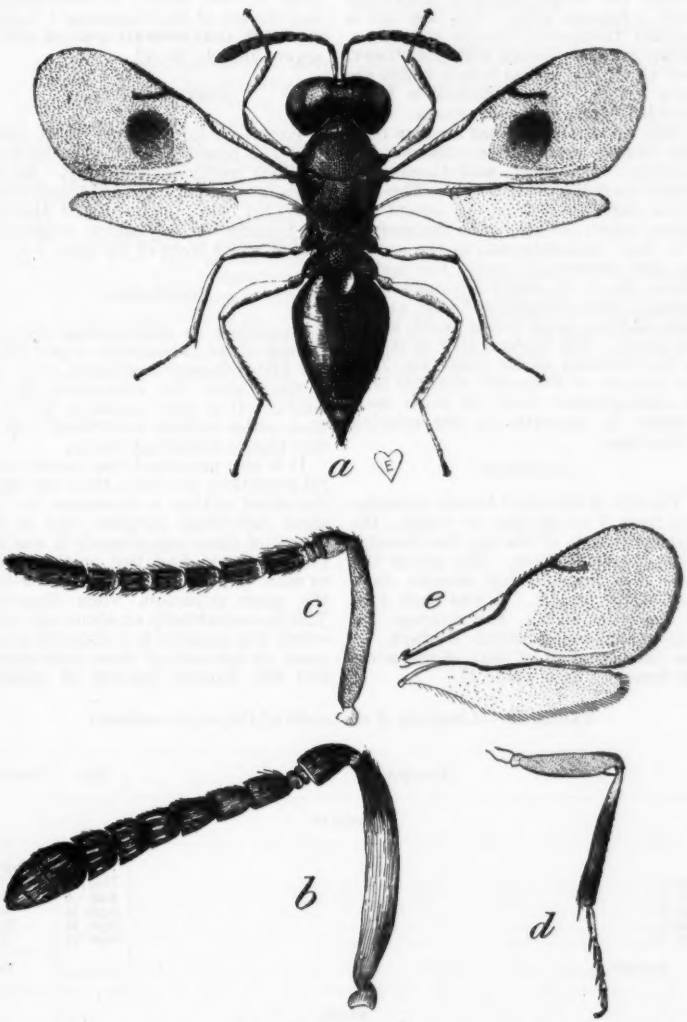

FIG. 2.—*Polyscelis modestus:* *a*, Adult female (actual length 2.3 mm.); *b*, antenna of female; *c*, antenna of male; *d*, median leg of male, showing femur, tibia, and tarsus; *e*, wings of male. All greatly enlarged.

wing. The head and the thorax are bronzy to brassy green. The clypeal region, the under side of the thorax, and the propodeum are tinged with blue. The antenna (fig. 2, *b*) is black, except the scape which is testaceous with a fuscous apex. The legs are a reddish testaceous, except the coxae, which are concolorous with the thorax, and the apical tarsal joints, which are dark brown. The abdomen is black with bronzy metallic reflections.

The male is somewhat smaller than the female and quite different in habitus. The head and thorax are bright metallic blue-green. The antenna (fig. 2, *c*) is fuscous, except the scape, which is very pale testaceous. The legs, including all of the coxae, are pale testaceous, except the middle tibiae (fig. 2, *d*), which are black with narrow, pale testaceous bands at the base, and the apical tarsal joints, which are black. The fuscous spot is absent in the forewing of the male (fig. 2, *e*) but this sex of *Polyscelis modestus* may be distinguished from the other male Hessian fly parasites by the coloring of the legs.

LONGEVITY

The life of the adult female parasites was from 7 to 39 days in length, the average length of life for five females being about 24 days. The life of the male was considerably shorter than that of the female. It was from 5 to 11 days in length, the average for four males being about 9 days, or less than one-half of that of the average female. (See Table II.)

SEX RATIO

Thirteen adults of this species have been reared from Hessian fly puparia collected in the field. Of these 13 specimens 6 were males, 6 were females, and the sex of the remaining 1 was unrecorded, thus indicating a sex ratio of approximately 50–50.

PARTHENOGENESIS

Experimental rearings have shown that it is possible for this species to reproduce parthenogenetically. In two cases in which unfertilized females were provided with unparasitized Hessian-fly puparia for oviposition, progeny resulted which were of the male sex.

OVIPOSITION

According to observations recorded during these laboratory experiments, the first oviposition occurred from 5 to 7 days after the emergence of the adults. It is very probable, however, that under natural conditions oviposition begins somewhat earlier.

It is also presumed that under natural conditions not more than one egg is deposited within a puparium by the same individual parasite; but in the course of these experiments it was not uncommon to find two or three eggs or more than one parasitic larva within the same puparium when dissected. This is undoubtedly an abnormal habit which the parasite is compelled to assume on account of close confinement and the limited number of puparia

TABLE II.—*Longevity of the adults of Polyscelis modestus*

Emerged	Died	Longevity
FEMALES		
		Days
May 8	May 15	7
July 11	Aug. 6	26
Aug. 8	Sept. 16	39
Aug. 8	Sept. 16	39
Sept. 13	Sept. 21	8
Average		23.8
MALES		
May 7	May 15	8
Aug. 1	Aug. 6	5
Sept. 10	Sept. 21	11
Sept. 5	Sept. 16	11
Average		8.75

provided each time for oviposition. In no case did more than one adult emerge from a puparium, although in possibly 10 or more cases more than one egg or more than one larva were found within a puparium.

EFFECT OF PARASITE ON HOST

In its attack upon the host the larva of *Polyscelis modestus* liquefies the contents of the Hessian fly larva as do other chalcidoid larvae of the Hessian fly parasites which have been studied. This process of liquefaction takes place rapidly, being almost completed in 24 hours after the parasite has attacked its host. A period of quiescence follows the attachment of the parasitic larva to its host and probably it is during this period that a digestive fluid which causes disintegration is injected by the primary parasitic larva. When the internal organs of the host are completely disintegrated, the liquefied contents are rapidly consumed by the parasite.

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SUMMARY

Polyscelis modestus Gahan, which was first discovered in 1915, has thus far proved to be a Hessian fly parasite of only minor importance.

Its present known distribution is confined to the southeastern and south central parts of Pennsylvania and the north central part of Maryland.

It attacks and destroys both the larvae and the pupae of the Hessian fly, as also the larvae and probably the eggs and pupae of its own species. It is occasionally hyperparasitic, probably on *Platygaster vernalis* (Myers).

Females reproducing parthenogenetically are arrhenotokous.

Before consumption by the parasite the internal contents of the host undergo a process of liquefaction, which probably is caused by the injection of a secretion by the primary parasitic larva.

LONGEVITY AND FECUNDITY OF BRUCHUS QUADRIMACULATUS FAB. AS INFLUENCED BY DIFFERENT FOODS [1]

By A. O. LARSON, *Assistant Entomologist*, and C. K. FISHER, *Junior Entomologist*, *Stored Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

It is remarkable how writers have refrained from making definite statements in regard to the feeding habits of adult weevils, *Bruchus quadrimaculatus*. The general assumption is that no food is required, and that as soon as the energy stored up by the larva is exhausted the adult dies. This is borne out by the fact that weevils of both sexes are smaller at the end of life than at the time of emergence; and the female shrinks to about one-third her former size. In warm weather this energy is used up more rapidly than in cooler weather. The insect therefore lives longer in a low temperature than in a high one, but does not produce more eggs during the long period of life than during the shorter period at the higher temperature.

The few known statements regarding the feeding habits of this weevil are not altogether in accord with one another. In speaking of *Bruchus chinensis*, which, he says, is typical of the other bruchids, Kunhikannan (*1*)[2] says: "The adults appear to take no food during their life." Wade (*5*), speaking of *B. quadrimaculatus*, says: "Weevils were bred in successive generations, * * * the adults taking neither food nor drink throughout their existence without apparent injury to them or effect upon their activities. Under these conditions adult females were kept alive for as many as 40 days." He says further: "That the adults will drink was demonstrated on several occasions when water was given them, of which they partook greedily. So far as could be learned, access to water did not stimulate their activities or prolong their life. That they feed can not be stated definitely; none were ever observed to do so, although it is quite possible that they may feed some on the green pods and foliage of the host plant." Sanborn (*4*), discussing the same weevil, says: "The adults are not as ravenous as the young or larvæ." Paddock and Reinhard (*3*) say: "The active feeding period is confined to the larval stage." In the same publication they say: "The cowpea weevil [the common name which they apply to *B. quadrimaculatus*] has not been observed to feed on any solid food in the adult stage * * *. In the field the adults feed almost exclusively upon nectar secreted by the nectaries located at the base of the green pods."

In confinement the length of life of the adult varies from a few days to a few weeks, depending on the temperature. In a warehouse the weevil gets no food, but finds conditions favorable for reproduction during the short time it lives. This is not the case in the field. There is a long period every summer during which there is no suitable place for oviposition in the field, but the crops become infested later. This would indicate one of three things: First, that infestation is caused by weevils which have emerged during the summer shortly before the crop is sufficiently matured for oviposition to take place; or, second, that the female weevil has the ability in the field to assimilate material which should normally go to form part of the quota of eggs, as is claimed to be the case with poultry, thus being sustained until she finds a suitable place to deposit the remainder of the eggs; or, third, that she consumed some kind of nourishment which prolongs her life. Experiments and observations indicate

[1] Received for publication July 26, 1924. Issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 305.

that in the great majority of instances the first is true. The second does not appear plausible, but the third may offer a solution for very light infestations in some cases and forms the basis for the experiments herein described.

SCOPE OF THE EXPERIMENTS

The present paper gives a summary of results of experiments conducted by the writers in 1923 at Alhambra, Calif. Four different sets of experiments beginning May 7 were carried on, three simultaneously and one later. Red Ripper cowpeas in glass vials 3 inches by ⅞ inch in size, provided with cork stoppers, were used throughout the experiments.

In experiment one, 100 pairs of newly emerged weevils were placed in vials, each of which contained 6 cowpeas. The vials were numbered consecutively from 1 to 100. Into each of the vials numbered 1, 5, 9, and every fourth vial to and including 97, was put a drop of water. Into each vial numbered 2, 6, 10, and every fourth vial to and including 98, was put a drop of honey. Vials, 3, 7, 11, etc., to and including 99, were each given a drop of saturated sugar water, while the remaining vials, 4, 8, 12, etc., contained only cowpeas and weevils, used as controls. All were examined daily, the cowpeas being replaced by others, the eggs recorded, and the water, sugar water, and honey being replenished as needed. Because of evaporation the water needed to be replenished daily and the honey and sugar solution less frequently. The date when the male or female died was also recorded.

Experiments two and three were similar in arrangement except that in experiment two only one male was placed in each vial, while in experiment three only one female was placed in each vial. Some of the females were known to have mated and others had had an opportunity to mate.

In experiment four only 24 females were taken, none of which had had an opportunity to mate. They were numbered and given food in the same manner as the others, there being only six individuals in each of the groups having water, honey, sugar water, or nothing, while in each of the other experiments there were 25 in each group.

The experiments were intended primarily to determine whether or not the presence of certain foods would aid the adult weevils to live longer than they ordinarily lived under normal warehouse conditions; in other words, to try to determine if food would support these short-lived insects while their host plants were becoming sufficiently developed for oviposition to occur. Whether adult weevils will live 3 months or less than 20 days is a vital question when trying to control them in the field. Together with the length of life as influenced by different kinds of foods this summary deals with the length of life as it is influenced by mating and reproduction, the number of eggs and their viability as influenced by the food and age of the parent female, and the asexual production of eggs. These data are related to the rate of increase as well as to the control of the weevils. It is thought that they will contribute something toward a more thorough knowledge of a very vital part of the life of this destructive and widespread species.

While the principal food of *Bruchus quadrimaculatus* in California is the black-eyed cowpea, it apparently breeds as freely in any other variety of cowpeas (*Vigna sinesis*) which may be available. Because the eggs can be seen more readily on Red Ripper cowpeas than on Black-eyes, the former were used throughout the experiments.

RESULTS OF EXPERIMENT 1

Table I presents the results of experiment 1. An examination shows readily that there was not only a great difference between the average length of life of the weevils receiving liquid and that of those receiving none, but it also shows that there was a corresponding difference in the number of eggs laid. The males and females without food lived an average of 17.48 and 17.72 days, respectively, with maxima of 22 and 27 days. Those given water lived an average of 10 days longer, or 27.24 days, with a maximum of 35 days for the males and 51 for the females. Those fed on honey averaged 27.76 days, or only a fraction of a day longer than those given water, but the maximum age for males was 60 and for females 44. Those fed on sugar water lived an average of 36.84 and 30.68 days for males and females, respectively, with maxima of 56 and 54 days. For both sexes the average length of life in each group having liquid was greater than the maximum for either sex without food; while the average life of the males receiving sugar water was more than twice that of those without food. Three insects, representing both sexes, lived twice as long as any weevil with-

out food. The group of weevils which lived the shortest length of time produced the smallest number of eggs. As the total length of life in all individuals of a group increased, the total number of eggs increased. The weevils receiving sugar water produced 49 per cent more eggs than those without food.

As was stated earlier, daily records were kept of the eggs laid by each female. These daily records are summarized in Table II, which shows the total number of eggs laid each day by the weevils of each group and the number and percentage of these eggs which hatched and emerged.

That the weevils which received no food were normal for weevils so treated is borne out by numerous observations from various authors. Wade (*5*) says that in Oklahoma "The number of eggs deposited by females varies from 11 to more than 100, 75 being about the average with favorable conditions obtaining." Courtney (*3*) in Texas found that during May, 1916, the average number of eggs laid by 30 females was 81, and during June the average number for 32 females was 88 eggs. Larson and Simmons (*2*) found that during September and October the average laid by 61 females was 88.52, the minimum number being 28 and the maximum 131. Throughout these experiments only weevils were used which were known to be newly emerged and which had not obtained food or deposited eggs. They were numbered consecutively, without selection as to size, color markings, or any other character, and were given their food after being numbered. The vials in which they were contained, the cowpeas on which they deposited, and their location in the laboratory with reference to light and temperature, were uniform for all groups of weevils; any difference in the length of life of the weevils and the number of eggs produced by each group is therefore directly attributable to the difference in the foods they were given.

TABLE I.—*Length of life of males and females and number of eggs deposited by 100 pairs of Bruchus quadrimaculatus given water, honey, sugar water, and nothing*

Weevils given water				Weevils given honey				Weevils given sugar water				Weevils without food or drink			
Pair No.	Longevity of—		Eggs laid	Pair No.	Longevity of—		Eggs laid	Pair No.	Longevity of—		Eggs laid	Pair No.	Longevity of—		Eggs laid
	Males	Females			Males	Females			Males	Females			Males	Females	
	Days	*Days*			*Days*	*Days*			*Days*	*Days*			*Days*	*Days*	
1	35	18	114	2	32	10	28	3	42	9	47	4	16	18	28
5	29	21	75	6	23	23	121	7	52	[a] 3	1	8	14	17	104
9	22	45	93	10	28	10	59	11	26	29	151	12	17	12	67
13	35	45	133	14	22	22	113	15	31	22	154	16	17	12	70
17	28	22	151	18	23	26	115	19	38	54	142	20	16	14	72
21	21	21	138	22	25	36	134	23	43	27	141	24	15	15	96
25	32	11	76	26	24	27	140	27	38	[b] 17	139	28	18	18	124
29	34	27	82	30	23	23	140	31	56	35	160	32	13	13	78
33	31	45	119	34	28	34	157	35	31	34	180	36	17	20	111
37	28	20	126	38	16	44	153	39	53	21	27	40	20	17	103
41	34	24	115	42	29	33	120	43	36	41	186	44	16	17	77
45	26	19	143	46	24	24	140	47	36	33	182	48	17	27	48
49	29	22	141	50	33	19	94	51	35	27	133	52	17	22	73
53	26	28	159	54	21	33	145	55	24	26	160	56	19	16	59
57	24	22	157	58	21	34	147	59	37	19	136	60	17	10	68
61	16	16	71	62	31	38	71	63	31	33	170	64	19	22	119
65	22	23	103	66	41	26	126	67	40	31	162	68	20	20	101
69	18	26	132	70	28	38	131	71	30	41	179	72	19	19	97
73	22	23	132	74	27	31	140	75	33	36	196	76	17	19	87
77	33	51	111	78	19	22	131	79	29	47	143	80	19	15	96
81	30	20	135	82	26	29	140	83	38	42	142	84	20	20	113
85	27	19	104	86	26	33	144	87	27	40	151	88	22	20	109
89	18	40	117	90	24	43	148	91	23	45	115	92	15	22	105
93	29	48	7	94	60	18	80	95	46	39	83	96	18	22	131
97	32	25	138	98	40	18	80	99	46	16	24	100	19	16	77
Total	681	681	2,872	-----	694	694	2,997	-----	921	767	3,304	-----	437	443	2,213
Aver.	27.24	27.24	114.88	-----	27.76	27.76	119.88	-----	36.84	30.68	132.16	-----	17.48	17.72	88.52
Max.	35	51	159	-----	60	44	157	-----	56	54	196	-----	22	27	131

[a] Female got stuck in the sugar water.

[b] Female accidentally killed.

TABLE II.—*Number of eggs laid each day by 100 pairs of Bruchus quadrimaculatus given water, honey, sugar water, and nothing; also the number and percentage of these eggs which hatched and emerged from each group*

Day	Water					Honey				
	Number of eggs	Number hatched	Per cent hatched	Number emerged	Per cent emerged	Number of eggs	Number hatched	Per cent hatched	Number emerged	Per cent emerged
1st	386	325	84.20	291	75.39	379	305	80.47	282	74.41
2d	330	263	79.70	228	69.09	335	275	82.09	255	76.12
3d	245	198	80.82	183	74.69	239	201	84.10	191	79.92
4th	195	159	81.54	141	72.31	179	135	75.42	126	70.39
5th	158	133	84.18	124	78.48	149	106	71.14	94	63.08
6th	163	136	83.44	127	77.91	156	101	64.74	95	60.90
7th	180	137	76.11	127	70.56	165	110	66.67	105	63.64
8th	108	86	79.63	82	75.93	144	91	63.19	85	59.03
9th	131	87	66.41	85	64.89	132	88	66.67	80	60.61
10th	117	81	69.23	77	65.81	114	66	57.89	62	54.39
11th	114	86	75.44	79	69.30	113	72	63.72	65	57.52
12th	114	82	71.93	73	64.04	103	65	63.11	58	56.31
13th	114	73	64.04	66	57.89	106	72	67.92	64	60.38
14th	109	77	70.64	65	59.63	104	71	68.27	63	60.58
15th	119	67	56.30	62	52.10	107	60	56.07	52	48.60
16th	84	31	36.90	25	29.76	102	63	61.76	55	53.92
17th	77	21	27.27	20	25.97	61	29	47.54	20	32.79
18th	47	10	21.28	7	14.89	63	34	53.97	23	36.51
19th	32	7	21.87	6	18.75	48	19	39.58	12	25.00
20th	21	1	4.76	1	4.76	46	23	50.00	18	39.13
21st	5	0				41	18	43.90	14	34.15
22d	5	0				28	11	39.29	6	21.43
23d	2	0				17	3	17.65	3	17.65
24th	0					17	9	52.94	5	29.41
25th	2	0				11	4	36.36	2	18.18
26th	1	0				8	2	25.00	1	12.50
27th	1	0				8	1	12.50	0	
28th	3	0				6	0			
29th	1	0				3	0			
30th	1	0				5	0			
31st	2	0				1	0			
32d	1	0				0				
33d	0					5	0			
34th	0					0				
35th	1	0				0				
36th	0					1	0			
37th	1	0				0				
38th	0					0				
39th	2	0				0				
40th	0					1	0			
41st	0					0				
42d	0					0				
43d	0					0				
44th	0					*0				
51st	*0									
54th										
Total or per cent	2,872	2,060	71.73	1,869	65.08	2,997	2,034	67.87	1,836	61.26

* Last 0 indicates date when last female died.

TABLE II.—*Number of eggs laid each day by 100 pairs of Bruchus quadrimaculatus given water, honey, sugar water, and nothing; also the number and percentage of these eggs which hatched and emerged from each group*—Continued

Day	Sugar water					Nothing				
	Number of eggs	Number hatched	Per cent hatched	Number emerged	Per cent emerged	Number of eggs	Number hatched	Per cent hatched	Number emerged	Per cent emerged
1st	313	264	84.34	237	75.72	417	327	78.42	294	70.50
2d	314	256	81.53	246	78.34	310	257	82.90	246	79.35
3d	221	172	77.83	164	74.21	225	164	72.89	147	65.33
4th	200	148	74.00	137	68.50	183	130	71.04	125	68.31
5th	153	115	75.16	109	71.24	149	119	79.87	107	71.81
6th	164	132	80.49	119	72.56	147	113	76.87	108	73.47
7th	143	121	84.62	115	80.42	132	106	80.30	101	76.52
8th	112	87	77.68	85	75.89	112	86	76.79	81	72.32
9th	126	97	76.98	94	74.60	85	57	67.06	52	61.18
10th	118	89	75.42	85	72.03	91	61	67.03	58	63.74
11th	116	83	71.55	73	62.93	89	58	65.17	51	57.30
12th	116	87	75.00	78	67.24	63	35	55.56	32	50.79
13th	129	94	72.87	89	68.99	66	47	71.21	46	69.70
14th	122	88	72.13	77	63.11	49	25	51.02	23	46.94
15th	125	93	74.40	81	64.80	47	27	57.45	25	53.19
16th	137	95	69.34	83	60.58	22	11	50.00	11	50.00
17th	102	59	57.84	51	50.00	12	4	33.33	4	33.33
18th	90	52	57.78	42	46.67	6	4	66.67	2	33.33
19th	79	45	56.96	38	48.10	4	0			
20th	70	34	48.57	30	42.86	0				
21st	58	31	53.45	25	43.10	2	0			
22d	44	20	45.45	18	40.91	2	0			
23d	45	21	46.67	18	40.00	0				
24th	22	9	40.91	5	22.73	0				
25th	33	12	36.36	7	21.21	0				
26th	24	6	25.00	4	16.67	0				
27th	19	1	5.26	1	5.26	0				
28th	16	3	18.75	2	12.50					
29th	12	2	16.67	1	8.33					
30th	12	0								
31st	10	0								
32d	3	2	66.67	2	66.67					
33d	30	5	16.67	3	10.00					
34th	9	0								
35th	4	2	50.00	2	50.00					
36th	6	0								
37th	2	0								
38th	2	0								
39th	0									
40th	0									
41st	0									
42d	2	0								
43d	1	0								
44th	0									
51st	0									
54th	*0									
Total or per cent	3,304	2,325	70.37	2,121	64.19	2,313	1,631	73.70	1,513	68.37

The weevils receiving water, honey, and sugar water laid 30, 35, and 49 per cent more eggs, respectively, than were laid by those which received nothing. From these eggs only 24, 21, and 40 per cent more weevils emerged than from those laid by the control group. There was a greater percentage of emergence from the eggs laid by the weevils which received no food than from those laid by either of the other groups, while the total emergence from each of the groups receiving food was considerably larger than that from the group receiving none. This difference in the percentage of emergence may be accounted for in two ways. First, it may have been accidental, because frequently when the eggs were being removed from the containers they would become smeared with sugar water, honey, or water. The probability that the eggs would become smeared was greatest in the case of the weevils which were given honey and least in the case of those given water. The percentage of emergence was lowest (67.87) for the former and highest (71.72) for the latter of the three groups fed. Second, the lower viability of the eggs laid late in the lives of the parent weevils may have caused the difference. A survey of Table II shows that the period of oviposition of the groups receiving food was much longer than that of the control group. It also shows that not only the number of eggs decreased toward the latter end of the weevil's life, but the viability also decreased. This is corroborated by Larson and Simmons' (*2*) record of 61 pairs.

Lack of space prevents the presentation of the records of individual weevils but these records show that of those without food 16 laid their maximum number of eggs on the first day, 7 on the second day, 1 on more than one day, and 1 on the fourth day. Of those given sugar water 10 laid the maximum number on the first day, 8 on the second, 2 on the third, 1 each on the fourth and sixth days, and 3 on more than one day. This tendency is carried through the groups, and accounts for the rapid and regular decrease in the number of eggs (Table II) for the group without food, and the relatively slow and irregular decrease in the group receiving sugar. It is interesting to note that the total number of eggs of each group dropped to less than 100 on the sixteenth, seventeenth, eighteenth, and ninth days for those given water, honey, sugar water, and nothing, respectively. The maximum number of eggs laid by one weevil during one day was 34—laid by a female without food. While we have frequently noted higher individual records than 34, it was 8 more than the maximum for any weevil receiving sugar water.

It is of economic importance as well as of scientific interest that the last eggs were laid on the thirty-ninth, fortieth, forty-third, and twenty-second days, respectively, and that the last eggs which produced weevils were laid on the twentieth, twenty-sixth, thirty-fifth, and eighteenth days by the weevils fed on water, honey, sugar water, and nothing, respectively. It appears, therefore, that weevils receiving sugar water will deposit viable eggs during twice as long a period of time as will weevils receiving no food.

The various foods apparently had no injurious or beneficial effect on the progeny. The emergence was uniform for all groups, the first emergence from eggs laid by each group being recorded in 53 days, or on June 29. The last emergence from the water-fed group occurred on July 31, while the date of last emergence from the sugar-fed group was August 2, the date of last emergence for the other groups being August 1. Thus the last emergence occurred 34 days later than the first, and 87 days after the first eggs were laid. The sexes were about equally divided, the 1,514 emerged weevils from the group not fed being 783 males and 731 females; the 1,870 from the water-fed group were 926 and 944, the 1,836 from the honey-fed group were 919 and 917, and those from the sugar-fed group were 1,077 males and 1,044 females; thus of the 7,341 emerged weevils 50.47 per cent were males.

RESULTS WITH 100 LONE MALES AND 100 LONE FEMALES

Because these weevils are polygamous and copulate freely it is of economic importance to know what, if any, effect the state of celibacy has on the length of life of the different sexes. Table III summarizes the length of life of 100 lone male weevils which were fed in the same manner as were the 100 pairs, and of the 100 males of the 100 pairs considered in connection with Table II.

TABLE III.—*Longevity of 100 lone males and 100 mating males given water, honey, sugar water, and nothing*

Food given	Longevity of lone males			Longevity of mating males		
	Maximum	Minimum	Average	Maximum	Minimum	Average
	Days	*Days*	*Days*	*Days*	*Days*	*Days*
Water	54	3	33.6	35	16	27.24
Honey	83	22	54.4	60	16	27.76
Sugar water	88	21	58.08	56	23	36.84
Nothing	48	20	30.96	22	13	17.48
Average			44.26			27.33

Table III shows that for both these groups of males, those which received no food lived the shortest average length of time, followed in order by those given water, honey, and sugar water. The maximum number of days lived by any individual of each group is in nearly the same ascending order. In each group the average length of time lived as well as the maximum for an individual is greater with the lone males than with the others. A lone male lived a maximum of 88 days while the maximum for a mating male was only 60 days. The average for all the unmated males was 44.27, compared with 27.33 for the other group.

The lone males and the lone females in Tables III and IV were not separated until some of them were known to have mated; in some instances, however, single individuals of each sex had emerged in separate containers. As it is known that these weevils normally copulate frequently this experiment demonstrated that a single mating was all that was necessary to fertilize the eggs laid during a long period of time. Of the group of lone females 92 per cent laid eggs. Only 73 per cent laid viable eggs. In order to determine whether the females were able to produce eggs without fertilization or whether a much larger percentage of the females had been fertilized than was believed to have been the case, another series of females, each individual of which had emerged alone in a container, was carried on. (Table V.)

Table IV summarizes the length of life of 100 lone females and the females from the 100 pairs discussed in connection with Table II, following the same method as that of Table III.

TABLE IV.—*Longevity of 100 lone females and 100 mating females given water, honey, sugar water, and nothing*

Food given	Longevity of lone females			Longevity of mating females		
	Maximum	Minimum	Average	Maximum	Minimum	Average
	Days	*Days*	*Days*	*Days*	*Days*	*Days*
Water	52	25	38.16	51	11	27.24
Honey	65	8	41.4	44	10	27.36
Sugar water	80	15	53.56	54	* 3	30.68
Nothing	50	18	27.8	27	10	17.72
Average			40.23			25.75

* Weevil got stuck in sugar water.

Table IV, following the general results of Tables I and III, shows that the average length of life of all females, lone, or mating, is shortest for the group receiving nothing, and increases for the groups receiving water, honey, and sugar water. For the lone females as with the lone males the maximum length of time lived by an individual of each group was shortest without food, longer when given water, still longer when receiving honey, and longest when fed sugar water. The lone females which received no food, although they produced an average of 62 eggs, lived an average of 10 days longer than the average number of days lived by the mating females. The lone females given water produced an average of 57 eggs and lived an average of 11 days longer than the corresponding mating females. Females receiving honey produced an average of 66 eggs and lived an average of 14 days longer than the mating females receiving the same food. The females receiving sugar water laid an average of 56 eggs each and lived an average of 23 days longer than the average for the corresponding mating females. Only in the group of lone females fed on honey did a weevil which had laid fertile eggs live as long as virgins in the same group. The maximum number of eggs laid by lone females were for nothing, water, honey, and sugar water, respectively, 110, 137, 116, and 148. For the average and maximum number of eggs laid by the mating females see Table I.

While the great majority of the viable eggs laid by the lone females were deposited early in the life of the females, some weevils emerged from eggs laid by the water-fed and honey-fed weevils when they were 24 days old. Weevils emerged from eggs laid by the sugar-water group and the group without food when they were 23 days and 18 days old, respectively.

RESULTS WITH VIRGIN FEMALES

The 24 virgin females studied in experiment 4 emerged July 16 in separate containers where no males emerged, and so had no chance to mate. Table V shows that the virgin females which did not receive food were unable to live as long as those receiving food. The virgin females lived longer than the mating females receiving the same treatment as to food (Table IV), but not as long as the lone females. The temperature during late July and August was much higher than earlier in the season, and the increased temperature undoubtedly shortened the lives of the last group of weevils. Although 17 eggs were laid by these weevils, they all failed to hatch, which would indicate that weevils of this species may deposit a small number of eggs without copulation having taken place, but that the eggs will not hatch.

TABLE V.—*Results of feeding experiments with 24 virgin females, Bruchus quadrimaculatus, fed on water, honey, sugar water, and nothing, 1923*

Fed on water			Fed on honey			Fed on sugar water			Not fed		
Number of weevils	Longevity	Eggs deposited	Number of weevils	Longevity	Eggs deposited	Number of weevils	Longevity	Eggs deposited	Number of weevils	Longevity	Eggs deposited
	Days			*Days*			*Days*			*Days*	
1	49	1	2	33	0	3	28	5	4	24	1
5	22	1	6	42	1	7	32	0	8	22	0
9	39	0	10	30	0	11	49	0	12	26	0
13	23	0	14	29	1	15	25	0	16	22	0
17	42	0	18	22	0	19	51	0	20	23	1
21	32	0	22	45	5	23	67	0	24	24	1
Total	207	2		201	7		252	5		141	3
Average	34.5	0.33		33.5	1.17		42.0	0.83		23.5	0.50
Maximum	49	1		45	5		67	5		26	1
Minimum	22	0		22	0		25	0		22	0

CONCLUSIONS

The knowledge that the average length of life of *Bruchus quadrimaculatus* is prolonged considerably in confinement by access to water and much more by access to sugar water suggests that the same species may be able to find a more suitable food in the field. Whether this food consists of only small particles of dew on the leaves, of nectar in the blossoms, of so-called honeydew on the leaves and stems, or of some other substance, remains to be learned. However, there is a decided difference between the problem of controlling weevils if they live less than 20 days on an average and 30 days as a maximum and the corresponding problem if they live an average of 50 or 60 days with 88 or more as a maximum. These problems will be discussed in another paper.

Weevils in their normal condition in the warehouse live a shorter time without food than when they have access to water or sweetened water.

The difference in the average length of life of the weevils receiving no food and those receiving water varied from less than 3 days with the lone males to 11 with the lone females. Access to water lengthened the lives of the pairs about 10 days. Sugar water lengthened their lives from 13 to 27 days as an average for different groups of weevils.

Access to water increases the average number of eggs laid by about 30 per cent, and access to sugar water increases the number of eggs about 50 per cent.

Food reduced the number of eggs laid during the first few days of oviposition, but lengthened the time over which eggs were laid. Viable eggs were laid over twice as great a period of time by weevils receiving sugar water as by those without food.

Frequent mating such as occurs normally under storage conditions reduced the length of life of both males and females. Females which mated only once during the first few hours after emergence deposited large numbers of fertile eggs.

Virgins deposited only small numbers of infertile eggs and in no case produced fertile eggs.

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A DOMINANT LETHAL CHLOROPHYLL MUTATION IN MAIZE [1]

By J. H. KEMPTON

Assistant Botanist, Office of Biophysical Investigations, Bureau of Plant Industry, United States Department of Agriculture

There are two outstanding facts with respect to the large number of heritable variations found in maize: First, practically all are degenerative with a lower survival value than normal plants, and second, they usually are recessive, being carried from generation to generation in latent form. These two facts bear a direct relation to each other, for if deleterious variations were not recessive they would be eliminated promptly from the stock at a rate proportional to their lower survival value. It seems clear, therefore, that the large number of recessive as compared with dominant variations is due largely to the fact that the recessive variations represent the accumulation of mutations through many generations, while the only dominant mutations found are those which have originated recently or which have no selective value. There is, of course, a gradual elimination of the recessive mutations also, the rate depending upon their productivity in a homozygous form, but this elimination is comparatively slow.

Recently, the writer has had an excellent illustration of the elimination of a dominant mutation. In a population of 50 plants of an F_1 between two varieties of maize, one of which had been inbred for eleven, the other seven, generations there appeared a single plant one-half of which was normal green in color, the other half being yellow, of a shade similar to the yellow seedling of Lindstrom.[2]

Neither parent of the cross from which this plant arose ever has produced yellow seedlings, and none of the sister plants showed the slightest tendency in this direction. The plant as a whole was a perfect example of a sectorial chimera. The chimeral nature appeared with the first leaf and extended throughout the entire plant. One-half of each leaf as well as one-half of the tassel was yellow, the affected portion being on the same side of the plant in each case. This division was apparent even on the central spike, where all the spikelets on one side were yellow and those on the other green. Two upper tassel branches were similar to the central spike, but the other branches of the tassel were divided equally, being either all green or all yellow. In appearance, the plant resembled the chimera reported by Khadilkar [3] except that in the case he reported the affected half was variegated yellow and white instead of self-yellow.

The plant was very weak and when mature was less than one-third the size of its normal sibs. The growth also was slower and the flowers matured about 10 days later than those of the normal plants. No ear was formed but pollen was produced abundantly.

Pollen was collected separately from the self-yellow and the self-green branches and as self-fertilization was not possible the pollen from the two sorts of spikelets was applied to two sister plants in an unrelated progeny which had been self-fertilized for 13 generations and which had never produced either albino or yellow seedlings.

Being inbred for such a long period, the resulting ears were not as large as could be desired, but 200 seedlings were grown from each, those having the green section of the chimera as a male parent giving only green seedlings while those with the yellow section of the chimera as a male parent gave 102 green and 98 yellow seedlings. This obviously is an approximation of the 1 : 1 ratio expected if the yellow portion of the plant was heterozygous for a dominant factor for the yellow chlorophyll disorder.

Most of the seedlings were grown out of doors in the ordinary manner but a few seeds were reserved for planting in greenhouse flats to provide a comparison with seedlings raised from the self-pollinated green sibs which survived in the field culture.

[1] Received for publication July 29, 1924 Issued January, 1925.

[2] LINDSTROM, E. W.—CHLOROPHYLL INHERITANCE IN MAIZE. N. Y. Cornell Agr. Exp. Sta. Mem. 13, 68 p., illus. 1918.

[3] KHADILKAR, T. R.—A SECTORIAL CHIMERA IN MAIZE. Jour. Heredity 12: 284-285. 1921.

The yellow seedlings were similar to the normal green seedlings in all respects except color. Those grown out-of-doors died after producing three or four leaves while the green plants grew normally without indication of chlorophyll disorders. Fifteen of these surviving green plants together with 18 of the plants having the green section of the chimera as a male parent were self-pollinated.

From these self-pollinated ears 4,489 seedlings were grown in the greenhouse, all being normal with no recurrence of the yellow character. In addition, the progeny of self-pollinated normal sibs of the chimera resulted in 547 plants all normal with respect to chlorophyll disorders.

Although these greenhouse seedlings were normal with respect to the yellow coloration they exhibited another type of chlorophyll disorder. This latter form, in extreme cases, resembled ordinary albinism but with imperceptible gradation from pure white to normal green. There was much variability between the progenies of different ears in this respect, some having 100 per cent of the plants affected, while in others less than 10 per cent showed the character to a noticeable degree. Except in the most extreme cases the plants recovered after the first four or five leaves developed and then remained normal.

It was thought at first that this second deficiency was related to the dominant yellow disorder indicating a general breakdown of chlorophyll, but subsequently unrelated progenies grown under the same conditions exhibited the phenomenon. Remnants of the ears planted in the field showed no evidence of this albinistic character, and it now seems probable that this character depends for its expression on some of the peculiar environmental factors of greenhouse culture.

At the time the seedlings were grown in the greenhouse the remainder of the F_1 hybrid seeds having the yellow section of the chimera as the male parent were planted, giving 12 green and 16 yellow seedlings. Contrary to their behavior in the field, the yellow seedlings raised in the greenhouse grew slowly and finally flowered, and although no seeds were produced, a small amount of pollen was shed. The growth of these seedlings had been so slow, however, that all the normal plants had matured long before the yellow plants flowered and only plants of the annual teosinte (*Euchlaena mexicana*) were available for crossing. Several crosses were made between yellow plants and this teosinte but only 40 seeds resulted. Of these 10 seedlings are now growing, 6 being yellow and 4 green. From this behavior it seems clear that the yellow half of the chimera was the result of a dominant mutation from green to yellow in one chromosome at a very early somatic cell division since it affected almost exactly one-half of the plant as well as the germ plasm.

Since plants lacking chlorophyll can not survive, the production of a further generation of this mutation was made possible only by the fortuitous circumstance that it involved but one-half of the plant. Had it occurred at an earlier cell division or previous to fertilization, the result would have been a single self-yellow seedling which would have perished without progeny.

As none of the yellow seedlings in the subsequent generation survived in the field, and as these were the only plants carrying this dominant mutation the character would have been automatically eliminated, precluding further study. It is believed that this is the only dominant mutation observed in maize within historic times and it furnishes evidence why such mutations have not been observed more frequently.

The fact that the chlorophyll deficiency observed in this case proved heritable raises the question as to whether similar deficiencies observed in Japonica plants would not also prove to be heritable if the pollen could be collected from the white areas.

There can be no question that on extreme Japonica plants the albino stripes often involve entire spikelets of the staminate inflorescence, an excellent illustration having been afforded in the case of a Japonica plant which produced an apogamous terminal panicle where each spikelet developed into a minature plant. Some of these minature plants were perfect albinos, others variegated, and still others, self-green. With the exception of the albinos, such plants can be propagated and it seems not unreasonable to infer that normal self-green plants could be produced from the recessive Japonica type by such means. The occurrence of albino plantlets strongly suggests that had these spikelets developed normally they would have produced pollen genetically albinistic and the occurrence of albino seedlings in the progeny of many commercial strains of Japonica may be due to this phenomenon.

SUMMARY

A sectorial chimera in which one-half of the plant was yellow, the other green, appeared in an F_1 maize hybrid. Pollen from the two halves of the plant was applied to normal green plants. The cross having the green side of the chimera as the male parent gave only green seedlings in F_1 and F_2 progeny, while the cross having as a male parent the yellow side of the chimera gave equal numbers of yellow and green plants in the F_1. The green plants gave only green F_2 and the yellow plants in the field died after producing three or four leaves. Yellow plants raised in the greenhouse reached maturity and produced pollen but no silks. The pollen from these yellow plants used on the annual teosinte, *Euchlaena mexicana*, resulted in an F_1 with approximately equal numbers of yellow and green seedlings.

It is concluded that the yellow character represents a dominant mutation, lethal under field culture. From the fact that the germ plasm proved to be of the same nature as the somatic tissue, it is suggested that the same phenomenon may be present in Japonica plants where the striping extends through the inflorescence.

THE RATE OF GROWTH OF GREEN AND ALBINO MAIZE SEEDLINGS[1]

By J. H. KEMPTON

Assistant Botanist, Biophysical Investigations, United States Department of Agriculture

For several years continuous records of elongation of many species of plants growing naturally in the field have been obtained by means of an auxanometer adapted to field use.[2] Chief among the plants measured is maize and from the hundreds of records obtained for this species it has become increasingly evident that much individuality exists in the responses of plants to meteorological changes even where such plants are related closely and are growing in near proximity. These differences in reaction between individuals in many cases must result from very small environmental fluctuations, though in others it seems more probable that the observed behavior is the result of inherent differences within the plants. In general, however, the elongation of all maize plants is accelerated by increased temperature, at least below 100° F. and probably above this temperature. The minimum rate is reached shortly after the minimum temperature, usually an hour or two before sunrise. In addition, maize plants seem not to be sensitive to changes in temperature or solar radiation which persist for less than 30 minutes.

In connection with the inherent differences between plants in their response to temperature and solar radiation it became of interest to compare the elongation rate of albino seedlings with that of their normal green sibs.

As is well known, there are several forms of albino seedlings in maize, the most extreme of which show no trace of chlorophyll. The growth of such seedlings should be comparable to that made by the sprouts of stored tubers or bulbs where the elongation results from the transfer of stored material. The latter type of elongation, however, is checked by light, whereas albino seedlings growing in sunlight continue to increase in size until the endosperm is exhausted. If solar radiation is a direct factor in the elongation of maize (for which there is some evidence) albino seedlings would be expected to react very differently from normal green plants since the practically pure white leaves would reflect a large part of the light. The reflection of light might be expected to result in a lower temperature for such plants but in reality there can be little difference since the leaves of normal green plants are maintained at practically air temperature through the effects of transpirations.

There is little difference in the size of normal and albino maize seedlings until three leaves have been produced, but after this the normal plants forge rapidly ahead while the albinos as rapidly decline. It is not unusual to find albino seedlings with four leaves and more rarely with five, but for the most part the leaves above the third are very much smaller than similar leaves of normal plants. Normal plants seem to derive little benefit from the material elaborated by the first few leaves unless the size of the root system is increased, though an inspection of the roots of albino and normal seedlings of the same age reveals no outstanding differences.

The accompanying figure is one of several very similar figures obtained from sister maize seedlings grown in the same hill in the field. The graph shows the hourly elongation rate of the second leaf in each case, the air temperature, and the solar radiation as measured by the differential thermograph devised by Briggs.[3]

The growth curves are remarkedly alike, the greatest difference consisting of 0.2 mm., being little larger than the experimental error. It is apparent from the figure that the maximum hourly rate of elongation is no greater in the green than in the albino plants, and that, like the green plants, the albinos have their maximum rate during the

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hours of daylight. Both plants show a rather closer agreement with radiation than with air temperature.

not so obviously dissimilar show very different reactions to variations in radiation and temperature. Such be-

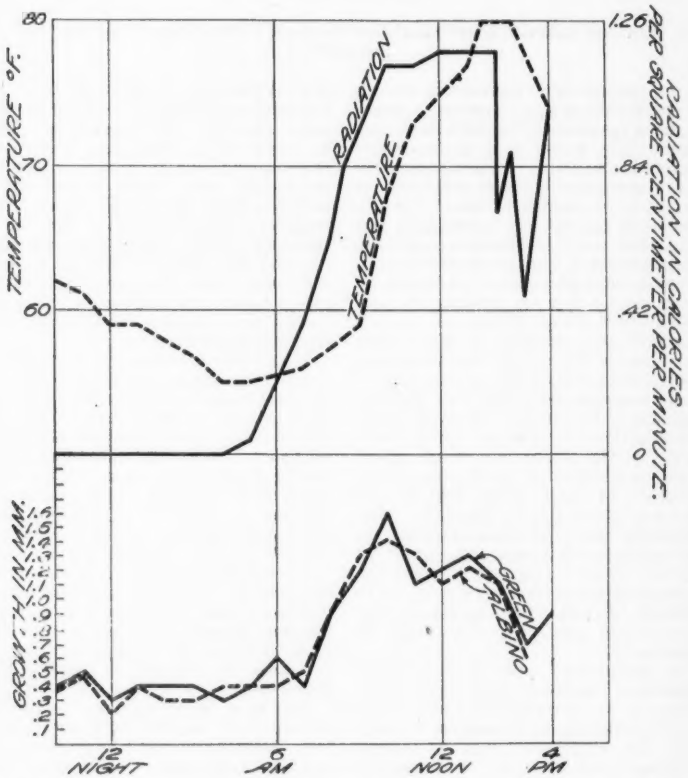

FIG. 1.—Hourly rate of growth of green and albino maize seedlings compared with air temperature and solar radiation

It seems rather remarkable that these sister maize plants differing so greatly in a major physiological characteristic should have such similar growth rates when in many other instances plants

havior shows that the response of the plant to air temperature and radiation is controlled by inherent factors not associated with the production of chlorophyll and photosynthesis.

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